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* * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *
FILE 'HOME' ENTERED AT 16:14:54 ON 06 DEC 2004
=> file caba caplus embase japio lifesci medline scisearch uspatfull
=> e frank glenn r/au
E1            8     FRANK GLENN/AU
E2            1     FRANK GLENN O/AU
E3           79 --> FRANK GLENN R/AU
E4           16     FRANK GLENN ROBERT/AU
E5            1     FRANK GLENN W/AU
E6            1     FRANK GLOCKNER/AU
E7            2     FRANK GLORIA/AU
E8           16     FRANK GLYNN H/AU
E9            2     FRANK GLYNN HENRY/AU
E10           1     FRANK GOERING/AU
E11           2     FRANK GOLIN/AU
E12           4     FRANK GOLLINSKI/AU
=> s e3-e4 and ige
L1           22 ("FRANK GLENN R"/AU OR "FRANK GLENN ROBERT"/AU) AND IGE
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2           22 DUP REM L1 (0 DUPLICATES REMOVED)
=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 22 ANSWERS - CONTINUE? Y/(N):y
L2   ANSWER 1 OF 22  USPATFULL on STN
AN     2004:273712  USPATFULL
TI     Method to detect   ***IgE***
IN       ***Frank, Glenn R.***  , Wellington, CO, UNITED STATES
       Porter, James P., Fort Collins, CO, UNITED STATES
       Rushlow, Keith E., Fort Collins, CO, UNITED STATES
       Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI     US 2004214209      A1   20041028
AI     US 2004-763400     A1   20040123 (10)
RLI    Division of Ser. No. US 2001-944277, filed on 30 Aug 2001, GRANTED, Pat.
       No. US 6682894 Division of Ser. No. US 1999-285873, filed on 31 Mar
       1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387,
       filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT     Utility
FS     APPLICATION
LREP   HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY,
       FORT COLLINS, CO, 80525
CLMN   Number of Claims: 10
ECL    Exemplary Claim: 106
DRWN   11 Drawing Page(s)
LN.CNT 1870
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention includes a method to detect   ***IgE***   using a
       human Fc epsilon receptor (Fc.sub..epsilon.R) to detect   ***IgE***
       antibodies in a biological sample from a cat dog, horse or mouse. The
       present invention also relates to kits to perform such methods.

L2   ANSWER 2 OF 22  USPATFULL on STN
AN     2003:264741  USPATFULL
TI     Novel ectoparasite saliva proteins and apparatus to collect such
       proteins
IN       ***Frank, Glenn R.***  , Wellington, CO, UNITED STATES
       Hunter, Shirley W., Ft. Collins, CO, UNITED STATES
       Wallenfels, Lynda, St. George, UT, UNITED STATES
PA     Heska Corporation. (U.S. corporation)
PI     US 2003185755      A1   20031002
AI     US 2002-271344     A1   20021014 (10)
RLI    Continuation of Ser. No. US 1997-809423, filed on 1 May 1997, ABANDONED
       A 371 of International Ser. No. WO 1995-US13200, filed on 6 Oct 1995,
       PENDING
DT     Utility
FS     APPLICATION
LREP   SHERIDAN ROSS PC, 1560 BROADWAY, SUITE 1200, DENVER, CO, 80202
CLMN   Number of Claims: 67
ECL    Exemplary Claim: 1
DRWN   15 Drawing Page(s)
LN.CNT 5498
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention includes a saliva protein collection apparatus capable of collecting ectoparasite saliva proteins substantially free of contaminating material. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

L2 ANSWER 3 OF 22 USPATFULL on STN
AN 2003:244340 USPATFULL
TI Method to detect dirofilaria immitis infection
IN Grieve, Robert B., Fort Collins, CO, UNITED STATES
Frank, Glenn R. , Wellington, CO, UNITED STATES
Mondesire, Roy R., Boulder, CO, UNITED STATES
Porter, James P., Fort Collins, CO, UNITED STATES
Wisnewski, Nancy, Fort Collins, CO, UNITED STATES
PI US 2003170749 A1 20030911
AI US 2002-150519 A1 20020517 (10)
RLI Division of Ser. No. US 1996-715628, filed on 18 Sep 1996, GRANTED, Pat. No. US 6391569
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 74
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 1953
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect D. immitis infection in a host animal using a D. immitis Di33 protein to detect anti-D. immitis Di33 antibodies in a bodily fluid of the animal. Also included is a method to detect D. immitis infection in a host animal using a D. immitis anti-Di33 protein to detect Di33 proteins in a bodily fluid of the animal. The present invention also relates to D. immitis detection kits that include either a Di33 protein or an anti-Di33 antibody; such kits also include a composition to detect an immunocomplex between the anti-Di33 antibody and D. immitis Di33 protein. The present invention also includes Di33 proteins, nucleic acid molecules encoding such proteins, as well as recombinant molecules and recombinant cells comprising such nucleic acid molecules, and anti-Di33 antibodies. Also included are methods to produce such proteins, nucleic acid molecules and antibodies.

L2 ANSWER 4 OF 22 USPATFULL on STN
AN 2003:155460 USPATFULL
TI Ectoparasite saliva proteins and apparatus to collect such proteins
IN Weber, Eric R., Ft. Collins, CO, United States
Hunter, Shirley Wu, Fort Collins, CO, United States
Frank, Glenn Robert , Wellington, CO, United States
Wallenfels, Lynda, St. George, UT, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6576238 B1 20030610
WO 9845408 19981015
AI US 1998-981799 19980827 (8)
WO 1997-US18669 19971015
RLI Continuation-in-part of Ser. No. WO 1997-US5959, filed on 10 Apr 1997 Continuation-in-part of Ser. No. US 1996-630822, filed on 10 Apr 1996, now patented, Pat. No. US 5840695 Continuation-in-part of Ser. No. US 1995-487001, filed on 7 Jun 1995, now patented, Pat. No. US 5795862 Continuation-in-part of Ser. No. US 1994-319590, filed on 7 Oct 1994, now patented, Pat. No. US 5646115
DT Utility
FS GRANTED
EXNAM Primary Examiner: Allen, Marianne P.

LREP Sheridan Ross P.C.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 6661
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

L2 ANSWER 5 OF 22 USPATFULL on STN
AN 2002:60937 USPATFULL
TI Method to detect ***IgE***
IN ***Frank, Glenn R.*** , Wellington, CO, UNITED STATES
Porter, James P., Fort Collins, CO, UNITED STATES
Rushlow, Keith E., Fort Collins, CO, UNITED STATES
Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI US 2002034771 A1 20020321
US 6682894 B2 20040127
AI US 2001-944277 A1 20010830 (9)
RLI Division of Ser. No. US 1999-285873, filed on 31 Mar 1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT Utility
FS APPLICATION
LREP Heska Corporation, Intellectual Property Dept., 1613 Prospect Parkway, Fort Collins, CO, 80525
CLMN Number of Claims: 105
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 2278
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon.R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L2 ANSWER 6 OF 22 USPATFULL on STN
AN 2002:310810 USPATFULL
TI Ectoparasite saliva proteins and apparatus to collect such proteins
IN Weber, Eric R., Ft. Collins, CO, United States
Hunter, Shirley Wu, Fort Collins, CO, United States
Sim, Gek-Kee, Fort Collins, CO, United States
Frank, Glenn Robert , Wellington, CO, United States
Wallenfels, Lynda, St. George, UT, United States4)
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6485968 B1 20021126
AI US 1998-4730 19980108 (9)
RLI Continuation of Ser. No. US 981799 Continuation-in-part of Ser. No. WO 1997-US5959, filed on 10 Apr 1997 Continuation-in-part of Ser. No. US 1996-630822, filed on 10 Apr 1996, now patented, Pat. No. US 5840695, issued on 24 Nov 1998 Continuation-in-part of Ser. No. WO 1995-US13200, filed on 6 Oct 1995 Continuation-in-part of Ser. No. US 1995-487001, filed on 7 Jun 1995, now patented, Pat. No. US 5795862, issued on 18 Aug 1998 Continuation-in-part of Ser. No. US 1995-487608, filed on 7 Jun 1995 Continuation-in-part of Ser. No. US 1994-319590, filed on 7 Oct 1994, now patented, Pat. No. US 5646115, issued on 8 Jul 1997
DT Utility
FS GRANTED
EXNAM Primary Examiner: Allen, Marianne P.
LREP Sheridan Ross P.C.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1

DRWN 10 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 6685
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

L2 ANSWER 7 OF 22 USPATFULL on STN
AN 2002:116009 USPATFULL
TI Method to detect Dirofilaria immitis infection
IN Grieve, Robert B., Fort Collins, CO, United States
Frank, Glenn R. , Wellington, CO, United States
Mondesire, Roy R., Boulder, CO, United States
Porter, James P., Fort Collins, CO, United States
Wisnewski, Nancy, Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6391569 B1 20020521
AI US 1996-715628 19960918 (8)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Minnifield, Nita
LREP Heska Corporation
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1576
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect D. immitis infection in a host animal using a D. immitis Di33 protein to detect anti-D. immitis Di33 antibodies in a bodily fluid of the animal. Also included is a method to detect D. immitis infection in a host animal using a D. immitis anti-Di33 protein to detect Di33 proteins in a bodily fluid of the animal. The present invention also relates to D. immitis detection kits that include either a Di33 protein or an anti-Di33 antibody; such kits also include a composition to detect an immunocomplex between the anti-Di33 antibody and D. immitis Di33 protein. The present invention also includes Di33 proteins, nucleic acid molecules encoding such proteins, as well as recombinant molecules and recombinant cells comprising such nucleic acid molecules, and anti-Di33 antibodies. Also included are methods to produce such proteins, nucleic acid molecules and antibodies.

L2 ANSWER 8 OF 22 USPATFULL on STN
AN 2001:190910 USPATFULL
TI Method to detect ***IgE***
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6309832 B1 20011030
AI US 1999-285873 19990331 (9)
RLI Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, now patented, Pat. No. US 5945294
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1536
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L2 ANSWER 9 OF 22 USPATFULL on STN
AN 2001:148088 USPATFULL
TI Feline Fc epsilon receptor alpha chain nucleic acid molecules
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6284881 B1 20010904
AI US 2000-515431 20000229 (9)
RLI Division of Ser. No. US 1998-5299, filed on 9 Jan 1998, now patented, Pat. No. US 6103494, issued on 15 Aug 2000 Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880, issued on 28 Sep 1999
DT Utility
FS GRANTED
EXNAM Primary Examiner: Gambel, Phillip; Assistant Examiner: Roark, Jessica H.
LREP Heska Corporation
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, compositions comprising such nucleic acid molecules, and methods to use such nucleic acid molecules to produce feline Fc epsilon receptor alpha chain proteins.

L2 ANSWER 10 OF 22 USPATFULL on STN
AN 2000:105679 USPATFULL
TI Feline Fc epsilon receptor alpha chain nucleic acid molecules, and uses thereof
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6103494 20000815
AI US 1998-5299 19980109 (9)
RLI Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880
DT Utility
FS Granted
EXNAM Primary Examiner: Mertz, Prema; Assistant Examiner: Hamud, Fozia
LREP Heska Corporation
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2779
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L2 ANSWER 11 OF 22 USPATFULL on STN
AN 2000:57620 USPATFULL
TI Method to detect canine ***IgE*** and kit therefor
IN ***Frank, Glenn R.*** , Wellington, CO, United States

Rushlow, Keith E., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6060326 20000509
AI US 1997-833488 19970407 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Gabel, Gailene R.
LREP Heska Corporation
CLMN Number of Claims: 38
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2232
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect canine ***IgE*** using a canine Fc epsilon receptor (Fc.sub..epsilon. R) to detect canine ***IgE*** antibodies in a biological sample from a canid. The present invention also relates to kits to perform such methods.

L2 ANSWER 12 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:844914 CAPLUS
DN 134:85060
TI Identification, cloning, and characterization of a major cat flea salivary allergen (Cte f 1)
AU McDermott, Martin J.; Weber, Eric; Hunter, Shirley; Stedman, Kim E.; Best, Elaine; ***Frank, Glenn R.*** ; Wang, Ruth; Escudero, Jean; Kuner, Jerry; McCall, Catherine
CS Heska Corporation, Fort Collins, CO, 80525, USA
SO Molecular Immunology (2000), 37(7), 361-375
CODEN: MOIMD5; ISSN: 0161-5890
PB Elsevier Science Ltd.
DT Journal
LA English
AB An 18 kDa protein isolated from saliva of the cat flea, Ctenocephalides felis, elicits a pos. intradermal skin test (IDST) in 100 and 80% of exptl. and clin. flea allergic dogs, resp. Using solid-phase enzyme-linked immuno assay (ELISA), this protein detected ***IgE*** in 100 and 80% of exptl. and clin. flea allergic dogs, resp. A cDNA (pFSI) encoding a full-length Cte f 1 protein was isolated from a C. felis salivary gland cDNA library, using a combination of PCR and hybridization screening. This cDNA is 658 bp in length, and contains an open reading frame of 528 bp. The open reading frame encodes a protein of 176 amino acids, consisting of an 18 amino acid signal sequence and a 158 amino acid mature protein. The calcd. mol. wt. and pI of the mature protein are 18,106 Da and 9.3, resp. The protein, named Cte f 1, is the first novel major allergen described for canine flea allergy. Recombinant Cte f 1 (rCte f 1) was expressed in Escherichia coli, Pichia pastoris and baculovirus infected Trichoplusia ni cells. Approx., 90% of the rCte f 1 expressed in E. coli accumulated in insol. inclusion bodies, which could be refolded to a sol. mixt. of disulfide isomers with partial ***IgE*** binding activity. Small quantities of an apparently correctly refolded form of rCte f 1, which had ***IgE*** binding activity equal to the native antigen, was isolated from the sol. fraction of E. coli cells. However, P. pastoris and baculovirus infected insect cells expressed and secreted a fully processed, correctly refolded and fully active form of rCte f 1. Mass spectrometry anal. of the active forms of rCte f 1 confirmed that eight intact disulfide bonds were present, matching the no. obsd. in the native allergen. The relative ability of rCte f 1 to bind ***IgE*** in the serum of flea allergic animals, produced in these three expression systems, matched that of the native allergen. Competition ELISA demonstrated that approx. 90% of the specific ***IgE*** binding to native Cte f 1 could be blocked by the different forms of rCte f 1.
RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 13 OF 22 USPATFULL on STN
AN 1999:117451 USPATFULL
TI Feline Fc epsilon receptor alpha chain proteins and therapeutic uses thereof
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States

Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5958880 19990928
AI US 1996-768964 19961219 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Kemmerer, Elizabeth
LREP Heska Corporation
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2759
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L2 ANSWER 14 OF 22 USPATFULL on STN
AN 1999:102683 USPATFULL
TI Method to detect ***IgE***
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5945294 19990831
AI US 1996-756387 19961126 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James; Assistant Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 77
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 2155
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L2 ANSWER 15 OF 22 USPATFULL on STN
AN 1999:89047 USPATFULL
TI Ectoparasite saliva proteins and apparatus to collect such proteins
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Hunter, Shirley Wu, Ft. Collins, CO, United States
Wallenfels, Lynda, Ft. Collins, CO, United States
PA Heska Corporation, Ft. Collins, CO, United States (U.S. corporation)
PI US 5932470 19990803
AI US 1998-5069 19980108 (9)
RLI Division of Ser. No. US 1996-630822, filed on 10 Apr 1996, now patented, Pat. No. US 5840695 which is a continuation-in-part of Ser. No. WO 1995-US13200, filed on 6 Oct 1995 which is a continuation-in-part of Ser. No. US 1995-487001, filed on 7 Jun 1995, now patented, Pat. No. US 5795862 And a continuation-in-part of Ser. No. US 1995-487608, filed on 7 Jun 1995 which is a continuation-in-part of Ser. No. US 1994-319590, filed on 7 Oct 1994, now patented, Pat. No. US 5646115
DT Utility
FS Granted
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Stole, Einar
LREP Ross P.C., Sheridan
CLMN Number of Claims: 9
ECL Exemplary Claim: 1

DRWN 12 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 6781
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention includes a saliva protein collection apparatus capable of collecting ectoparasite saliva proteins substantially free of contaminating material. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

L2 ANSWER 16 OF 22 USPATFULL on STN
AN 1999:83583 USPATFULL
TI Ectoparasite saliva proteins and apparatus to collect such proteins
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Hunter, Shirley Wu, Ft. Collins, CO, United States
Wallenfels, Lynda, Ft. Collins, CO, United States
PA Heska Corporation, Ft. Collins, CO, United States (U.S. corporation)
PI US 5927230 19990727
AI US 1996-711905 19960912 (8)
RLI Division of Ser. No. US 1994-319590, filed on 7 Oct 1994, now patented, Pat. No. US 5646115
DT Utility
FS Granted
EXNAM Primary Examiner: Price, Thomas
LREP Sheridan Ross, P.C.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 3771
AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention includes a saliva protein collection apparatus capable of collecting ectoparasite saliva proteins substantially free of contaminating material. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

L2 ANSWER 17 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:685117 CAPLUS
DN 129:314987
TI Canine Fc epsilon receptor and allergen to detect canine ***IgE***
IN ***Frank, Glenn Robert*** ; Rushlow, Keith E.
PA Heska Corporation, USA
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9845707	A1	19981015	WO 1998-US6774	19980406
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI,				

CM, GA, GN, ML, MR, NE, SN, TD, TG

	US 6060326	A	20000509	US 1997-833488	19970407
	AU 9867964	A1	19981030	AU 1998-67964	19980406
PRAI	US 1997-833488	A	19970407		
	WO 1998-US6774	W	19980406		

AB The present invention includes a method to detect canine ***IgE*** using a canine Fc epsilon receptor (Fc.epsilon.R) to detect canine ***IgE*** antibodies in a biol. sample from a canine. A method comprises contacting immobilized allergen with sample to form allergen-***IgE*** complexes, followed by contacting with immobilized Fc.epsilon.R for quantitating ***IgE*** and for diagnosing allergy. The allergen is derived from fungi, trees, weeds, shrubs, grasses, wheat, corn, soybean, rice, eggs, milk, cheese, bovine, poultry, swine, sheep, yeast, fleas, flies, mosquitoes, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs and ticks. The present invention also relates to kits to perform such methods.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 18 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:424343 CAPLUS
DN 129:94477
TI Feline Fc epsilon receptor alpha chain nucleic acids and proteins and diagnostic and therapeutic uses thereof
IN ***Frank, Glenn Robert*** ; Porter, James P.; Rushlow, Keith E.; Wassom, Donald L.; Weber, Eric R.
PA Heska Corp., USA
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9827208	A1	19980625	WO 1997-US23244	19971216
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5958880	A	19990928	US 1996-768964	19961219
	AU 9853841	A1	19980715	AU 1998-53841	19971216
	EP 950104	A1	19991020	EP 1997-950976	19971216
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002500507	T2	20020108	JP 1998-527923	19971216
	CA 2273855	C	20030527	CA 1997-2273855	19971216
	CA 2273855	AA	19980625		
	US 6103494	A	20000815	US 1998-5299	19980109
	US 6284881	B1	20010904	US 2000-515431	20000229
PRAI	US 1996-768964	A	19961219		
	WO 1997-US23244	W	19971216		
	US 1998-5299	A3	19980109		

AB The present invention relates to feline Fc.epsilon. receptor .alpha. chain nucleic acid mols., proteins encoded by such nucleic acid mols., antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compns. comprising such proteins, nucleic acid mols., antibodies and/or inhibitory compds. as well as the use of such therapeutic compns. to mediate Fc.epsilon. receptor-mediated biol. responses.

L2 ANSWER 19 OF 22 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:197685 CAPLUS
DN 128:281707
TI Method to detect Dirofilaria immitis infection
IN Grieve, Robert B.; ***Frank, Glenn R.*** ; Mondesire, Roy R.; Porter,

James P.; Wisnewski, Nancy
PA Heska Corporation, USA
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9812563	A1	19980326	WO 1997-US16535	19970918
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 6391569	B1	20020521	US 1996-715628	19960918
	CA 2266428	AA	19980326	CA 1997-2266428	19970918
	AU 9743537	A1	19980414	AU 1997-43537	19970918
	EP 934529	A1	19990811	EP 1997-941677	19970918
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2001502896	T2	20010306	JP 1998-514859	19970918
	US 2003170749	A1	20030911	US 2002-150519	20020517
PRAI	US 1996-715628	A	19960918		
	WO 1997-US16535	W	19970918		

AB The present invention includes a method to detect D. immitis infection in a host animal using a D. immitis Di33 protein to detect anti-D. immitis Di33 antibodies in a bodily fluid of the animal. Also included is a method to detect D. immitis infection in a host animal using a D. immitis anti-Di33 protein to detect Di33 proteins in a bodily fluid of the animal. The present invention also relates to D. immitis detection kits that include either a Di33 protein or an anti-Di33 antibody; such kits also include a compn. to detect an immunocomplex between the anti-Di33 antibody and D. immitis Di33 protein. The present invention also includes Di33 proteins, nucleic acid mols. encoding such proteins, as well as recombinant mols. and recombinant cells comprising such nucleic acid mols., and anti-Di33 antibodies. Also included are methods to produce such proteins, nucleic acid mols. and antibodies.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 20 OF 22 USPATFULL on STN
AN 1998:147412 USPATFULL
TI Ectoparasite saliva proteins and apparatus to collect such proteins
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Hunter, Shirley Wu, Ft. Collins, CO, United States
Wallenfels, Lynda, St. George, UT, United States
PA Heska Corporation, Ft. Collins, CO, United States (U.S. corporation)
PI US 5840695 19981124
AI US 1996-630822 19960410 (8)
RLI Continuation-in-part of Ser. No. US 1995-487001, filed on 7 Jun 1995, now patented, Pat. No. US 5795862 which is a continuation-in-part of Ser. No. US 1995-487608, filed on 7 Jun 1995 which is a continuation-in-part of Ser. No. US 1994-319590, filed on 7 Oct 1994, now patented, Pat. No. US 5646115
DT Utility
FS Granted
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Stole, Einar
LREP Sheridan Ross P.C.
CLMN Number of Claims: 41
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 6531
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention includes a saliva protein collection apparatus capable

of collecting ectoparasite saliva proteins substantially free of contaminating material. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

L2 ANSWER 21 OF 22 USPATFULL on STN
AN 1998:98886 USPATFULL
TI Ectoparasite saliva proteins and apparatus to collect such proteins
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Hunter, Shirley Wu, Ft. Collins, CO, United States
Wallenfels, Lynda, Ft. Collins, CO, United States
PA Heska Corporation, Ft. Collins, CO, United States (U.S. corporation)
PI US 5795862 19980818
AI US 1995-487001 19950607 (8)
RLI Continuation-in-part of Ser. No. US 1994-319590, filed on 7 Oct 1994, now patented, Pat. No. US 5646115
DT Utility
FS Granted
EXNAM Primary Examiner: Jacobson, Dian C.
LREP Sherifan Ross P.C.
CLMN Number of Claims: 29
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 4678
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention includes a saliva protein collection apparatus capable of collecting ectoparasite saliva proteins substantially free of contaminating material. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

L2 ANSWER 22 OF 22 USPATFULL on STN
AN 97:59173 USPATFULL
TI Ectoparasite saliva proteins and apparatus to collect such proteins
IN ***Frank, Glenn R.*** , Wellington, CO, United States
Hunter, Shirley Wu, Ft. Collins, CO, United States
Wallenfels, Lynda, Ft. Collins, CO, United States
PA Heska Corporation, Ft. Collins, CO, United States (U.S. corporation)
PI US 5646115 19970708
AI US 1994-319590 19941007 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Jacobson, Dian C.
LREP Sheridan Ross & McIntosh
CLMN Number of Claims: 33
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 3822
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to a novel product and method for isolating ectoparasite saliva proteins, and a novel product and method for detecting and/or treating allergic dermatitis in an animal. The present invention includes a saliva protein collection apparatus capable of collecting ectoparasite saliva proteins substantially free of contaminating material. The present invention also relates to ectoparasite saliva proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to obtain such proteins and

to use such proteins to identify animals susceptible to or having allergic dermatitis. The present invention also includes therapeutic compositions comprising such proteins and their use to treat animals susceptible to or having allergic dermatitis.

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=> e porter james p/au
E1            5      PORTER JAMES M/AU
E2            2      PORTER JAMES N/AU
E3           68 --> PORTER JAMES P/AU
E4            2      PORTER JAMES PAUL/AU
E5           38      PORTER JAMES R/AU
E6            2      PORTER JAMES RICHARD/AU
E7            4      PORTER JAMES S/AU
E8           10      PORTER JAMES T/AU
E9            1      PORTER JAMES THOMAS/AU
E10           1      PORTER JAMES V/AU
E11          10      PORTER JAMES W/AU
E12           4      PORTER JAMES WINSTON/AU

=> s e3-e4 and ige
L3           14 ("PORTER JAMES P"/AU OR "PORTER JAMES PAUL"/AU) AND IGE

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4           14 DUP REM L3 (0 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 14 ANSWERS - CONTINUE? Y/(N):y


L4   ANSWER 1 OF 14  USPATFULL on STN
AN     2004:273712  USPATFULL
TI     Method to detect   ***IgE***
IN     Frank, Glenn R., Wellington, CO, UNITED STATES
           ***Porter, James P.***  , Fort Collins, CO, UNITED STATES
       Rushlow, Keith E., Fort Collins, CO, UNITED STATES
       Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI     US 2004214209      A1   20041028
AI     US 2004-763400     A1   20040123 (10)
RLI    Division of Ser. No. US 2001-944277, filed on 30 Aug 2001, GRANTED, Pat.
       No. US 6682894 Division of Ser. No. US 1999-285873, filed on 31 Mar
       1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387,
       filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT     Utility
FS     APPLICATION
LREP   HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY,
       FORT COLLINS, CO, 80525
CLMN   Number of Claims: 10
ECL    Exemplary Claim: 106
DRWN   11 Drawing Page(s)
LN.CNT 1870
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention includes a method to detect   ***IgE***   using a
       human Fc epsilon receptor (Fc.sub..epsilon.R) to detect   ***IgE***
       antibodies in a biological sample from a cat dog, horse or mouse. The
       present invention also relates to kits to perform such methods.

L4   ANSWER 2 OF 14  USPATFULL on STN
AN     2003:244340  USPATFULL
TI     Method to detect dirofilaria immitis infection
IN     Grieve, Robert B., Fort Collins, CO, UNITED STATES
       Frank, Glenn R., Wellington, CO, UNITED STATES
       Mondesire, Roy R., Boulder, CO, UNITED STATES
           ***Porter, James P.***  , Fort Collins, CO, UNITED STATES
       Wisnewski, Nancy, Fort Collins, CO, UNITED STATES
PI     US 2003170749      A1   20030911
AI     US 2002-150519     A1   20020517 (10)
RLI    Division of Ser. No. US 1996-715628, filed on 18 Sep 1996, GRANTED, Pat.
       No. US 6391569
DT     Utility
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FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 74
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 1953
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect D. immitis infection in a host animal using a D. immitis Di33 protein to detect anti-D. immitis Di33 antibodies in a bodily fluid of the animal. Also included is a method to detect D. immitis infection in a host animal using a D. immitis anti-Di33 protein to detect Di33 proteins in a bodily fluid of the animal. The present invention also relates to D. immitis detection kits that include either a Di33 protein or an anti-Di33 antibody; such kits also include a composition to detect an immunocomplex between the anti-Di33 antibody and D. immitis Di33 protein. The present invention also includes Di33 proteins, nucleic acid molecules encoding such proteins, as well as recombinant molecules and recombinant cells comprising such nucleic acid molecules, and anti-Di33 antibodies. Also included are methods to produce such proteins, nucleic acid molecules and antibodies.

L4 ANSWER 3 OF 14 USPATFULL on STN
AN 2002:60937 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, UNITED STATES
Porter, James P. , Fort Collins, CO, UNITED STATES
Rushlow, Keith E., Fort Collins, CO, UNITED STATES
Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI US 2002034771 A1 20020321
US 6682894 B2 20040127
AI US 2001-944277 A1 20010830 (9)
RLI Division of Ser. No. US 1999-285873, filed on 31 Mar 1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT Utility
FS APPLICATION
LREP Heska Corporation, Intellectual Property Dept., 1613 Prospect Parkway, Fort Collins, CO, 80525
CLMN Number of Claims: 105
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 2278
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon.R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L4 ANSWER 4 OF 14 USPATFULL on STN
AN 2002:116009 USPATFULL
TI Method to detect Dirofilaria immitis infection
IN Grieve, Robert B., Fort Collins, CO, United States
Frank, Glenn R., Wellington, CO, United States
Mondesire, Roy R., Boulder, CO, United States
Porter, James P. , Fort Collins, CO, United States
Wisnewski, Nancy, Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6391569 B1 20020521
AI US 1996-715628 19960918 (8)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Minnifield, Nita
LREP Heska Corporation
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1576
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect D. immitis infection

in a host animal using a D. immitis Di33 protein to detect anti-D. immitis Di33 antibodies in a bodily fluid of the animal. Also included is a method to detect D. immitis infection in a host animal using a D. immitis anti-Di33 protein to detect Di33 proteins in a bodily fluid of the animal. The present invention also relates to D. immitis detection kits that include either a Di33 protein or an anti-Di33 antibody; such kits also include a composition to detect an immunocomplex between the anti-Di33 antibody and D. immitis Di33 protein. The present invention also includes Di33 proteins, nucleic acid molecules encoding such proteins, as well as recombinant molecules and recombinant cells comprising such nucleic acid molecules, and anti-Di33 antibodies. Also included are methods to produce such proteins, nucleic acid molecules and antibodies.

L4 ANSWER 5 OF 14 USPATFULL on STN
AN 2001:190910 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P. , Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6309832 B1 20011030
AI US 1999-285873 19990331 (9)
RLI Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, now patented, Pat. No. US 5945294
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1536
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L4 ANSWER 6 OF 14 USPATFULL on STN
AN 2001:148088 USPATFULL
TI Feline Fc epsilon receptor alpha chain nucleic acid molecules
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P. , Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6284881 B1 20010904
AI US 2000-515431 20000229 (9)
RLI Division of Ser. No. US 1998-5299, filed on 9 Jan 1998, now patented, Pat. No. US 6103494, issued on 15 Aug 2000 Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880, issued on 28 Sep 1999
DT Utility
FS GRANTED
EXNAM Primary Examiner: Gambel, Phillip; Assistant Examiner: Roark, Jessica H.
LREP Heska Corporation
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, compositions comprising such nucleic acid molecules, and methods to use such nucleic acid molecules to produce feline Fc epsilon receptor alpha chain proteins.

L4 ANSWER 7 OF 14 USPATFULL on STN
AN 2000:105679 USPATFULL

TI Feline Fc epsilon receptor alpha chain nucleic acid molecules, and uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P. , Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6103494 20000815
AI US 1998-5299 19980109 (9)
RLI Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880
DT Utility
FS Granted
EXNAM Primary Examiner: Mertz, Prema; Assistant Examiner: Hamud, Fozia
LREP Heska Corporation
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2779
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L4 ANSWER 8 OF 14 USPATFULL on STN
AN 1999:117451 USPATFULL
TI Feline Fc epsilon receptor alpha chain proteins and therapeutic uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P. , Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5958880 19990928
AI US 1996-768964 19961219 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Kemmerer, Elizabeth
LREP Heska Corporation
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2759
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L4 ANSWER 9 OF 14 USPATFULL on STN
AN 1999:102683 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P. , Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5945294 19990831

AI US 1996-756387 19961126 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James; Assistant Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 77
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 2155
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L4 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:424343 CAPLUS
DN 129:94477
TI Feline Fc epsilon receptor alpha chain nucleic acids and proteins and diagnostic and therapeutic uses thereof
IN Frank, Glenn Robert; ***Porter, James P.*** ; Rushlow, Keith E.; Wassom, Donald L.; Weber, Eric R.
PA Heska Corp., USA
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9827208	A1	19980625	WO 1997-US23244	19971216
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5958880	A	19990928	US 1996-768964	19961219
	AU 9853841	A1	19980715	AU 1998-53841	19971216
	EP 950104	A1	19991020	EP 1997-950976	19971216
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002500507	T2	20020108	JP 1998-527923	19971216
	CA 2273855	C	20030527	CA 1997-2273855	19971216
	CA 2273855	AA	19980625		
	US 6103494	A	20000815	US 1998-5299	19980109
	US 6284881	B1	20010904	US 2000-515431	20000229
PRAI	US 1996-768964	A	19961219		
	WO 1997-US23244	W	19971216		
	US 1998-5299	A3	19980109		

AB The present invention relates to feline Fc.epsilon. receptor .alpha. chain nucleic acid mols., proteins encoded by such nucleic acid mols., antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compns. comprising such proteins, nucleic acid mols., antibodies and/or inhibitory compds. as well as the use of such therapeutic compns. to mediate Fc.epsilon. receptor-mediated biol. responses.

L4 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:388688 CAPLUS
DN 129:66836
TI Method to detect ***IgE***
IN Frank, Robert Glenn; ***Porter, James P.*** ; Rushlow, Keith E.; Wassom, Donald L.
PA Heska Corporation, USA
SO PCT Int. Appl., 71 pp.
CODEN: PIXXD2

DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9823964	A1	19980604	WO 1997-US21651	19971124
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5945294	A	19990831	US 1996-756387	19961126
	CA 2270868	AA	19980604	CA 1997-2270868	19971124
	AU 9874114	A1	19980622	AU 1998-74114	19971124
	EP 943097	A1	19990922	EP 1997-949625	19971124
	EP 943097	B1	20030730		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2001507792	T2	20010612	JP 1998-526731	19971124
	AT 246361	E	20030815	AT 1997-949625	19971124
	ES 2205266	T3	20040501	ES 1997-949625	19971124
	US 6309832	B1	20011030	US 1999-285873	19990331
	AU 769954	B2	20040212	AU 2001-57951	20010809
	US 2002034771	A1	20020321	US 2001-944277	20010830
	US 6682894	B2	20040127		
	US 2004214209	A1	20041028	US 2004-763400	20040123
PRAI	US 1996-756387	A	19961126		
	AU 1998-74114	A3	19971124		
	WO 1997-US21651	W	19971124		
	US 1999-285873	A3	19990331		
	US 2001-944277	A3	20010830		

AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.epsilon.R) to detect ***IgE*** antibodies in a biol. sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods. The kits comprise an allergen common to all regions of the United States and a human Fc.epsilon. receptor mol.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:197685 CAPLUS
DN 128:281707
TI Method to detect Dirofilaria immitis infection
IN Grieve, Robert B.; Frank, Glenn R.; Mondesire, Roy R.; ***Porter, James*** *** P.*** ; Wisnewski, Nancy
PA Heska Corporation, USA
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9812563	A1	19980326	WO 1997-US16535	19970918
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 6391569	B1	20020521	US 1996-715628	19960918
	CA 2266428	AA	19980326	CA 1997-2266428	19970918
	AU 9743537	A1	19980414	AU 1997-43537	19970918
	EP 934529	A1	19990811	EP 1997-941677	19970918
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, SI, LT, LV, FI, RO

	JP 2001502896	T2	20010306	JP 1998-514859	19970918
	US 2003170749	A1	20030911	US 2002-150519	20020517
PRAI	US 1996-715628	A	19960918		
	WO 1997-US16535	W	19970918		

AB The present invention includes a method to detect D. immitis infection in a host animal using a D. immitis Di33 protein to detect anti-D. immitis Di33 antibodies in a bodily fluid of the animal. Also included is a method to detect D. immitis infection in a host animal using a D. immitis anti-Di33 protein to detect Di33 proteins in a bodily fluid of the animal. The present invention also relates to D. immitis detection kits that include either a Di33 protein or an anti-Di33 antibody; such kits also include a compn. to detect an immunocomplex between the anti-Di33 antibody and D. immitis Di33 protein. The present invention also includes Di33 proteins, nucleic acid mols. encoding such proteins, as well as recombinant mols. and recombinant cells comprising such nucleic acid mols., and anti-Di33 antibodies. Also included are methods to produce such proteins, nucleic acid mols. and antibodies.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1993:668528 CAPLUS
DN 119:268528
TI Humanization of an antibody directed against ***IgE***
AU Presta, Leonard G.; Lahr, Steven J.; Shields, Robert L.; ***Porter,*** *** James P.*** ; Gorman, Cornelia M.; Fendly, Brian M.; Jardieu, Paula M.
CS Dep. Protein Eng., Genentech, Inc., South San Francisco, CA, 94080, USA
SO Journal of Immunology (1993), 151(5), 2623-32
CODEN: JOIMA3; ISSN: 0022-1767
DT Journal
LA English
AB ***IgE*** antibodies bind to specific high-affinity receptors on mast cells, leading to mast cell degranulation and release of mediators, such as histamine, which produce symptoms assocd. with allergy. Hence, anti-***IgE*** antibodies that block binding of ***IgE*** to its high-affinity receptor are of potential therapeutic value in the treatment of allergy. These antibodies also must not bind to ***IgE*** once it is bound to the receptor because this would trigger histamine release. This study describes the humanization of a murine antibody, MaE11, with these characteristics. Variants of the humanized antibody were evaluated to probe the importance of framework residues on antibody binding and to det. which charged residues in the CDR interacted with ***IgE*** . Only five changes in human framework residues were required to provide for binding comparable to that of the original murine antibody.

L4 ANSWER 14 OF 14 USPATFULL on STN
AN 89:12811 USPATFULL
TI Method for the detection of equine infectious anemia and other retrovirus infections using a competitive enzyme-linked immunoabsorbent assay and reagents useful in the same
IN ***Porter, James P.*** , Oakland, CA, United States
Matsushita, Tatsuo, Fort Collins, CO, United States
Hesterberg, Lyndal K., Fort Collins, CO, United States
PA Fermenta Animal Health Company, Kansas City, MO, United States (U.S. corporation)
PI US 4806467 19890221
AI US 1985-789910 19851021 (6)
DT Utility
FS Granted
EXNAM Primary Examiner: Rosen, Sam
LREP Sughrue, Mion, Zinn, Macpeak, and Seas
CLMN Number of Claims: 29
ECL Exemplary Claim: 1,26
DRWN No Drawings
LN.CNT 885
AB The present invention relates to a method of detecting either antibody or antigen in the serum of horses infected with equine infectious anemia using a competitive enzyme-linked immunoabsorbent assay technique and reagents useful in such an assay. The competitive enzyme-linked immunoabsorbent assay incorporates a purified virus antigen conjugate

and a monoclonal antibody specific for the virus antigen as both the reacting and competing components. Alternatively, the competitive enzyme-linked immunoabsorbent assay incorporates a purified virus antigen and a monoclonal antibody conjugate specific for the viral antigen as both reacting and competing components. This invention also relates to detecting antigen and antibody found in other retrovirus infections such as Acquired Immunodeficiency Syndrome in humans.

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=> e rushlow keith e/au
E1            7     RUSHLOW KEITH/AU
E2            1     RUSHLOW KEITH A/AU
E3           57 --> RUSHLOW KEITH E/AU
E4            1     RUSHLOW KEITH EDWARD/AU
E5            1     RUSHLOW LEO M J/AU
E6            2     RUSHLOW MICHELLE M B/AU
E7            1     RUSHLOW PAUL S/AU
E8           41     RUSHLOW W/AU
E9           18     RUSHLOW W J/AU
E10           1     RUSHLOW WALTER/AU
E11           2     RUSHLOW WALTER J/AU
E12           1     RUSHLOW WALTER JAMES/AU

=> s e3-e4 and IgE
L5           11 ("RUSHLOW KEITH E"/AU OR "RUSHLOW KEITH EDWARD"/AU) AND IGE

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6           11 DUP REM L5 (0 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):y

L6   ANSWER 1 OF 11  USPATFULL on STN
AN     2004:273712  USPATFULL
TI     Method to detect   ***IgE***
IN     Frank, Glenn R., Wellington, CO, UNITED STATES
       Porter, James P., Fort Collins, CO, UNITED STATES
           ***Rushlow, Keith E.***  , Fort Collins, CO, UNITED STATES
       Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI     US 2004214209      A1   20041028
AI     US 2004-763400     A1   20040123 (10)
RLI    Division of Ser. No. US 2001-944277, filed on 30 Aug 2001, GRANTED, Pat.
       No. US 6682894 Division of Ser. No. US 1999-285873, filed on 31 Mar
       1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387,
       filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT     Utility
FS     APPLICATION
LREP   HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY,
       FORT COLLINS, CO, 80525
CLMN   Number of Claims: 10
ECL    Exemplary Claim: 106
DRWN   11 Drawing Page(s)
LN.CNT 1870
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention includes a method to detect   ***IgE***   using a
       human Fc epsilon receptor (Fc.sub..epsilon.R) to detect   ***IgE***
       antibodies in a biological sample from a cat dog, horse or mouse. The
       present invention also relates to kits to perform such methods.

L6   ANSWER 2 OF 11  USPATFULL on STN
AN     2002:60937  USPATFULL
TI     Method to detect   ***IgE***
IN     Frank, Glenn R., Wellington, CO, UNITED STATES
       Porter, James P., Fort Collins, CO, UNITED STATES
           ***Rushlow, Keith E.***  , Fort Collins, CO, UNITED STATES
       Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI     US 2002034771      A1   20020321
       US 6682894         B2   20040127
AI     US 2001-944277     A1   20010830 (9)
RLI    Division of Ser. No. US 1999-285873, filed on 31 Mar 1999, GRANTED, Pat.
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No. US 6309832 Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT Utility
FS APPLICATION
LREP Heska Corporation, Intellectual Property Dept., 1613 Prospect Parkway, Fort Collins, CO, 80525
CLMN Number of Claims: 105
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 2278
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon.R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L6 ANSWER 3 OF 11 USPATFULL on STN
AN 2001:190910 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E. , Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6309832 B1 20011030
AI US 1999-285873 19990331 (9)
RLI Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, now patented, Pat. No. US 5945294
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1536
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L6 ANSWER 4 OF 11 USPATFULL on STN
AN 2001:148088 USPATFULL
TI Feline Fc epsilon receptor alpha chain nucleic acid molecules
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E. , Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6284881 B1 20010904
AI US 2000-515431 20000229 (9)
RLI Division of Ser. No. US 1998-5299, filed on 9 Jan 1998, now patented, Pat. No. US 6103494, issued on 15 Aug 2000 Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880, issued on 28 Sep 1999
DT Utility
FS GRANTED
EXNAM Primary Examiner: Gambel, Phillip; Assistant Examiner: Roark, Jessica H.
LREP Heska Corporation
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, compositions comprising such nucleic acid molecules, and methods to use such nucleic acid molecules to produce feline Fc epsilon receptor alpha chain proteins.

L6 ANSWER 5 OF 11 USPATFULL on STN
AN 2000:105679 USPATFULL
TI Feline Fc epsilon receptor alpha chain nucleic acid molecules, and uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E. , Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6103494 20000815
AI US 1998-5299 19980109 (9)
RLI Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880
DT Utility
FS Granted
EXNAM Primary Examiner: Mertz, Prema; Assistant Examiner: Hamud, Fozia
LREP Heska Corporation
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2779
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L6 ANSWER 6 OF 11 USPATFULL on STN
AN 2000:57620 USPATFULL
TI Method to detect canine ***IgE*** and kit therefor
IN Frank, Glenn R., Wellington, CO, United States
Rushlow, Keith E. , Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6060326 20000509
AI US 1997-833488 19970407 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Gabel, Gailene R.
LREP Heska Corporation
CLMN Number of Claims: 38
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2232
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect canine ***IgE*** using a canine Fc epsilon receptor (Fc.sub..epsilon. R) to detect canine ***IgE*** antibodies in a biological sample from a canid. The present invention also relates to kits to perform such methods.

L6 ANSWER 7 OF 11 USPATFULL on STN
AN 1999:117451 USPATFULL
TI Feline Fc epsilon receptor alpha chain proteins and therapeutic uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E. , Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5958880 19990928
AI US 1996-768964 19961219 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Kemmerer, Elizabeth

LREP Heska Corporation
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2759
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L6 ANSWER 8 OF 11 USPATFULL on STN
AN 1999:102683 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E. , Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5945294 19990831
AI US 1996-756387 19961126 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James; Assistant Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 77
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 2155
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L6 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:685117 CAPLUS
DN 129:314987
TI Canine Fc epsilon receptor and allergen to detect canine ***IgE***
IN Frank, Glenn Robert; ***Rushlow, Keith E.***
PA Heska Corporation, USA
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9845707	A1	19981015	WO 1998-US6774	19980406
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 6060326	A	20000509	US 1997-833488	19970407
	AU 9867964	A1	19981030	AU 1998-67964	19980406
PRAI	US 1997-833488	A	19970407		
	WO 1998-US6774	W	19980406		

AB The present invention includes a method to detect canine ***IgE*** using a canine Fc epsilon receptor (Fc.epsilon.R) to detect canine ***IgE*** antibodies in a biol. sample from a canine. A method comprises contacting immobilized allergen with sample to form allergen-***IgE*** complexes, followed by contacting with immobilized

Fc.epsilon.R for quantitating ***IgE*** and for diagnosing allergy. The allergen is derived from fungi, trees, weeds, shrubs, grasses, wheat, corn, soybean, rice, eggs, milk, cheese, bovine, poultry, swine, sheep, yeast, fleas, flies, mosquitoes, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs and ticks. The present invention also relates to kits to perform such methods.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:424343 CAPLUS
DN 129:94477
TI Feline Fc epsilon receptor alpha chain nucleic acids and proteins and diagnostic and therapeutic uses thereof
IN Frank, Glenn Robert; Porter, James P.; ***Rushlow, Keith E.*** ; Wassom, Donald L.; Weber, Eric R.
PA Heska Corp., USA
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9827208	A1	19980625	WO 1997-US23244	19971216
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5958880	A	19990928	US 1996-768964	19961219
	AU 9853841	A1	19980715	AU 1998-53841	19971216
	EP 950104	A1	19991020	EP 1997-950976	19971216
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002500507	T2	20020108	JP 1998-527923	19971216
	CA 2273855	C	20030527	CA 1997-2273855	19971216
	CA 2273855	AA	19980625		
	US 6103494	A	20000815	US 1998-5299	19980109
	US 6284881	B1	20010904	US 2000-515431	20000229
PRAI	US 1996-768964	A	19961219		
	WO 1997-US23244	W	19971216		
	US 1998-5299	A3	19980109		

AB The present invention relates to feline Fc.epsilon. receptor .alpha. chain nucleic acid mols., proteins encoded by such nucleic acid mols., antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compns. comprising such proteins, nucleic acid mols., antibodies and/or inhibitory compds. as well as the use of such therapeutic compns. to mediate Fc.epsilon. receptor-mediated biol. responses.

L6 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:388688 CAPLUS
DN 129:66836
TI Method to detect ***IgE***
IN Frank, Robert Glenn; Porter, James P.; ***Rushlow, Keith E.*** ; Wassom, Donald L.
PA Heska Corporation, USA
SO PCT Int. Appl., 71 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9823964	A1	19980604	WO 1997-US21651	19971124

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          W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK,
              EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ,
              LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL,
              PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ,
              VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
          RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR,
              GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,
              GN, ML, MR, NE, SN, TD, TG
     US 5945294           A     19990831    US 1996-756387          19961126
     CA 2270868           AA    19980604    CA 1997-2270868         19971124
     AU 9874114           A1    19980622    AU 1998-74114           19971124
     EP 943097            A1    19990922    EP 1997-949625          19971124
     EP 943097            B1    20030730
          R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
              IE, SI, LT, LV, FI, RO
     JP 2001507792        T2    20010612    JP 1998-526731          19971124
     AT 246361            E     20030815    AT 1997-949625          19971124
     ES 2205266           T3    20040501    ES 1997-949625          19971124
     US 6309832           B1    20011030    US 1999-285873          19990331
     AU 769954            B2    20040212    AU 2001-57951           20010809
     US 2002034771        A1    20020321    US 2001-944277          20010830
     US 6682894           B2    20040127
     US 2004214209        A1    20041028    US 2004-763400          20040123
PRAI US 1996-756387       A     19961126
     AU 1998-74114        A3    19971124
     WO 1997-US21651      W     19971124
     US 1999-285873       A3    19990331
     US 2001-944277       A3    20010830
AB   The present invention includes a method to detect   ***IgE***   using a
     human Fc epsilon receptor (Fc.epsilon.R) to detect   ***IgE***
     antibodies in a biol. sample from a cat, a dog, or a horse.  The present
     invention also relates to kits to perform such methods.  The kits comprise
     an allergen common to all regions of the United States and a human
     Fc.epsilon. receptor mol.
RE.CNT  5     THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> e wassom donald l/au
E1          279     WASSOM D L/AU
E2            2     WASSOM D L */AU
E3           35 --> WASSOM DONALD L/AU
E4            1     WASSOM E E/AU
E5            6     WASSOM J/AU
E6           24     WASSOM J J/AU
E7           68     WASSOM J S/AU
E8            1     WASSOM JACK/AU
E9            1     WASSOM JAMES J/AU
E10           1     WASSOM JAMES JOSEPH/AU
E11           1     WASSOM JOHN/AU
E12          12     WASSOM JOHN S/AU

=> s e1-e3 and ige
L7           19 ("WASSOM D L"/AU OR "WASSOM D L *"/AU OR "WASSOM DONALD L"/AU)
                AND IGE

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8           14 DUP REM L7 (5 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 14 ANSWERS - CONTINUE? Y/(N):y

L8   ANSWER 1 OF 14  USPATFULL on STN
AN     2004:273712  USPATFULL
TI     Method to detect   ***IgE***
IN     Frank, Glenn R., Wellington, CO, UNITED STATES
       Porter, James P., Fort Collins, CO, UNITED STATES
       Rushlow, Keith E., Fort Collins, CO, UNITED STATES
           ***Wassom, Donald L.***  , Fort Collins, CO, UNITED STATES
PI     US 2004214209       A1    20041028
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AI US 2004-763400 A1 20040123 (10)
RLI Division of Ser. No. US 2001-944277, filed on 30 Aug 2001, GRANTED, Pat. No. US 6682894 Division of Ser. No. US 1999-285873, filed on 31 Mar 1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 10
ECL Exemplary Claim: 106
DRWN 11 Drawing Page(s)
LN.CNT 1870
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon.R) to detect ***IgE*** antibodies in a biological sample from a cat dog, horse or mouse. The present invention also relates to kits to perform such methods.

L8 ANSWER 2 OF 14 USPATFULL on STN
AN 2002:60937 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, UNITED STATES
Porter, James P., Fort Collins, CO, UNITED STATES
Rushlow, Keith E., Fort Collins, CO, UNITED STATES
Wassom, Donald L. , Fort Collins, CO, UNITED STATES
PI US 2002034771 A1 20020321
US 6682894 B2 20040127
AI US 2001-944277 A1 20010830 (9)
RLI Division of Ser. No. US 1999-285873, filed on 31 Mar 1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT Utility
FS APPLICATION
LREP Heska Corporation, Intellectual Property Dept., 1613 Prospect Parkway, Fort Collins, CO, 80525
CLMN Number of Claims: 105
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 2278
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon.R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L8 ANSWER 3 OF 14 USPATFULL on STN
AN 2001:190910 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L. , Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6309832 B1 20011030
AI US 1999-285873 19990331 (9)
RLI Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, now patented, Pat. No. US 5945294
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1536
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L8 ANSWER 4 OF 14 USPATFULL on STN
AN 2001:148088 USPATFULL
TI Feline Fc epsilon receptor alpha chain nucleic acid molecules
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L. , Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6284881 B1 20010904
AI US 2000-515431 20000229 (9)
RLI Division of Ser. No. US 1998-5299, filed on 9 Jan 1998, now patented, Pat. No. US 6103494, issued on 15 Aug 2000 Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880, issued on 28 Sep 1999
DT Utility
FS GRANTED
EXNAM Primary Examiner: Gambel, Phillip; Assistant Examiner: Roark, Jessica H.
LREP Heska Corporation
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, compositions comprising such nucleic acid molecules, and methods to use such nucleic acid molecules to produce feline Fc epsilon receptor alpha chain proteins.

L8 ANSWER 5 OF 14 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AN 2001:200464 SCISEARCH
GA The Genuine Article (R) Number: 405RE
TI Anti-storage mite ***IgE*** in human sera detached with the recombinant human Fc epsilon RI alpha
AU Stedman K E (Reprint); Hunter S W; McCall C A; ***Wassom D L***
CS Heska Corp, Ft Collins, CO USA
CYA USA
SO JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (FEB 2001) Vol. 107, No. 2, Supp. [S], pp. S123-S123. MA 408.
Publisher: MOSBY, INC, 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA.
ISSN: 0091-6749.
DT Conference; Journal
LA English
REC Reference Count: 0

L8 ANSWER 6 OF 14 USPATFULL on STN
AN 2000:105679 USPATFULL
TI Feline Fc epsilon receptor alpha chain nucleic acid molecules, and uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L. , Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6103494 20000815
AI US 1998-5299 19980109 (9)
RLI Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880
DT Utility
FS Granted
EXNAM Primary Examiner: Mertz, Prema; Assistant Examiner: Hamud, Fozia
LREP Heska Corporation
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2779
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L8 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1999:659581 CAPLUS
DN 131:285405
TI Method to detect biologically active, allergen-specific immunoglobulins
IN De Weck, Alain J.; ***Wassom, Donald L.***
PA Heska Corporation, USA
SO PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9951988	A1	19991014	WO 1999-US7530	19990406
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2328079	AA	19991014	CA 1999-2328079	19990406
	AU 9933845	A1	19991025	AU 1999-33845	19990406
	EP 1068535	A1	20010117	EP 1999-915297	19990406
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 1998-81089P	P	19980408		
	US 1998-99776P	P	19980910		
	WO 1999-US7530	W	19990406		

AB The present invention includes a method to detect a biol. active, allergen-specific Ig using a Fc epsilon receptor (Fc.epsilon.R) mol. Such a method can detect biol. active, allergen-specific Igs not detectable by a process using anti- ***IgE*** antibodies. The present invention also relates to kits to perform such methods. The present invention also includes a heat stable, biol. active, allergen-specific Ig.
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 8 OF 14 USPATFULL on STN
AN 1999:117451 USPATFULL
TI Feline Fc epsilon receptor alpha chain proteins and therapeutic uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L. , Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5958880 19990928
AI US 1996-768964 19961219 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Kemmerer, Elizabeth
LREP Heska Corporation
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2759
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to feline Fc epsilon receptor alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate Fc epsilon receptor-mediated biological responses.

L8 ANSWER 9 OF 14 USPATFULL on STN
AN 1999:102683 USPATFULL
TI Method to detect ***IgE***
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L. , Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5945294 19990831
AI US 1996-756387 19961126 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James; Assistant Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 77
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 2155
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.sub..epsilon. R) to detect ***IgE*** antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

L8 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:424343 CAPLUS
DN 129:94477
TI Feline Fc epsilon receptor alpha chain nucleic acids and proteins and diagnostic and therapeutic uses thereof
IN Frank, Glenn Robert; Porter, James P.; Rushlow, Keith E.; ***Wassom,*** *** Donald L.*** ; Weber, Eric R.
PA Heska Corp., USA
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9827208	A1	19980625	WO 1997-US23244	19971216
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5958880	A	19990928	US 1996-768964	19961219
	AU 9853841	A1	19980715	AU 1998-53841	19971216
	EP 950104	A1	19991020	EP 1997-950976	19971216
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002500507	T2	20020108	JP 1998-527923	19971216
	CA 2273855	C	20030527	CA 1997-2273855	19971216
	CA 2273855	AA	19980625		
	US 6103494	A	20000815	US 1998-5299	19980109
	US 6284881	B1	20010904	US 2000-515431	20000229
PRAI	US 1996-768964	A	19961219		
	WO 1997-US23244	W	19971216		
	US 1998-5299	A3	19980109		

AB The present invention relates to feline Fc.epsilon. receptor .alpha. chain nucleic acid mols., proteins encoded by such nucleic acid mols., antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect ***IgE*** using such proteins and antibodies. Also included in the present invention are therapeutic compns. comprising such proteins, nucleic acid mols., antibodies and/or inhibitory compds. as well as the use of such therapeutic compns. to mediate Fc.epsilon. receptor-mediated biol. responses.

L8 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:388688 CAPLUS
DN 129:66836
TI Method to detect ***IgE***
IN Frank, Robert Glenn; Porter, James P.; Rushlow, Keith E.; ***Wassom,*** *** Donald L.***
PA Heska Corporation, USA
SO PCT Int. Appl., 71 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9823964	A1	19980604	WO 1997-US21651	19971124
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5945294	A	19990831	US 1996-756387	19961126
	CA 2270868	AA	19980604	CA 1997-2270868	19971124
	AU 9874114	A1	19980622	AU 1998-74114	19971124
	EP 943097	A1	19990922	EP 1997-949625	19971124
	EP 943097	B1	20030730		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2001507792	T2	20010612	JP 1998-526731	19971124
	AT 246361	E	20030815	AT 1997-949625	19971124
	ES 2205266	T3	20040501	ES 1997-949625	19971124
	US 6309832	B1	20011030	US 1999-285873	19990331
	AU 769954	B2	20040212	AU 2001-57951	20010809
	US 2002034771	A1	20020321	US 2001-944277	20010830
	US 6682894	B2	20040127		
	US 2004214209	A1	20041028	US 2004-763400	20040123
PRAI	US 1996-756387	A	19961126		
	AU 1998-74114	A3	19971124		
	WO 1997-US21651	W	19971124		
	US 1999-285873	A3	19990331		
	US 2001-944277	A3	20010830		

AB The present invention includes a method to detect ***IgE*** using a human Fc epsilon receptor (Fc.epsilon.R) to detect ***IgE*** antibodies in a biol. sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods. The kits comprise an allergen common to all regions of the United States and a human Fc.epsilon. receptor mol.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 12 OF 14 CABA COPYRIGHT 2004 CABI on STN
AN 1999:9211 CABA
DN 19982219806
TI The in vitro diagnosis of flea bite hypersensitivity: flea saliva vs. whole-flea extracts
AU Cook, C. A.; Stedman, K. E.; Frank, G. R.; ***Wassom, D. L.*** ; Kwochka, K. W. [EDITOR]; Willemse, T. [EDITOR]; Tscharner, C. von [EDITOR]
CS Heska Corporation, Fort Collins, Colorado, USA.
SO Advances in veterinary dermatology: volume 3. Proceedings of the Third

World Congress of Veterinary Dermatology, Edinburgh, Scotland, 11-14 September, 1996, (1998) pp. 494-495.
Publisher: Butterworth-Heinemann Ltd. Oxford
Price: Conference paper; Book chapter .
Meeting Info.: Advances in veterinary dermatology: volume 3. Proceedings of the Third World Congress of Veterinary Dermatology, Edinburgh, Scotland, 11-14 September, 1996.
ISBN: 0-7506-3443-X
CY United Kingdom
DT Journal
LA English
ED Entered STN: 19990112
Last Updated on STN: 19990112

L8 ANSWER 13 OF 14 CABA COPYRIGHT 2004 CABI on STN DUPLICATE 1
AN 1998:174465 CABA
DN 19982217049
TI In vitro measurement of canine and feline ***IgE*** : a review of Fc[epsilon]R1[alpha]-based assays for detection of allergen-reactive ***IgE***
AU ***Wassom, D. L.*** ; Grieve, R. B.
CS Heska Corporation, 1825 Sharp Point Drive, Fort Collins, Colorado, 80525, USA.
SO Veterinary Dermatology, (1998) Vol. 9, No. 3, pp. 173-178. 6 ref.
ISSN: 0959-4493
DT Journal
LA English
ED Entered STN: 19981111
Last Updated on STN: 19981111

L8 ANSWER 14 OF 14 CABA COPYRIGHT 2004 CABI on STN DUPLICATE 2
AN 91:13800 CABA
DN 19910868020
TI Evidence for differential induction of helper T cell subsets during Trichinella spiralis infection
AU Pond, L.; ***Wassom, D. L.*** ; Hayes, C. E.
CS C.E. Hayes, Department of Biochemistry, University of Wisconsin, Madison, WI 53706, USA.
SO Journal of Immunology (Baltimore), (1989) Vol. 143, No. 12, pp. 4232-4237. 35 ref.
ISSN: 0022-1767
DT Journal
LA English
ED Entered STN: 19941101
Last Updated on STN: 19941101
AB The H-2-compatible mouse strains, AKR and B10.BR, exhibit disparate responses to infection with T. spiralis. The resistant AKR mice expel intestinal adult worms faster than susceptible B10.BR mice. Antibody and lymphokine responses were tested in these strains. With respect to antibody responses, the B10.BR mice had 3- to 10-fold more serum ***IgE*** and T. spiralis-specific IgG1 and IgA than AKR mice. The B10.BR mice also had greater numbers of IgG and IgA plaque-forming cells than AKR mice. In contrast, AKR mice produced T. spiralis-specific IgG2a, whereas the B10.BR mice did not. The antibody response kinetics of these strains were similar. Lymphokine secretion was analysed after restimulating lymphocytes in vitro with T. spiralis antigen. The AKR mesenteric lymph node cells produced more IFN-[gamma] and less IL-4 than the B10.BR mesenteric lymph node cells. The B10.BR splenocytes produced more IL-4 than the AKR splenocytes, although splenocyte IFN-[gamma] production was not different. The kinetics of IL-4 production also differed between the 2 strains. In summary, resistant AKR mice produced more IFN-[gamma] and T. spiralis-specific IgG2a than susceptible B10.BR mice, which produced more IL-4, ***IgE*** , and T. spiralis-specific IgG1. These results are consistent with differential activation of Th cell subsets in T. spiralis-infected AKR and B10.BR mice.

=> s human and Fc?
L9 106736 HUMAN AND FC?

=> s l9 and fc epsilon receptor?

```
L10        888 L9 AND FC EPSILON RECEPTOR?

=> d 888 kwic

L10  ANSWER 888 OF 888  USPATFULL on STN
SUMM   . . .  of N-linked oligosaccharide to IgE binding factors for the
       selective formation of IgE suppressive factors. It suppresses
       IgE-induced expression of   ***Fc***  -  ***epsilon***
         ***receptors***   on lymphocytes; the factor has a molecular weight of
       15-16 kilodaltons, and it binds to monoclonal antibodies against
       lipomodulin, Uede.  .  .  pgs. 1286-1294 (1984). Wallner, et al., in
       Nature, Vol. 320, pgs. 77-81 (1986) report the cloning, sequencing and
       expression of   ***human***   lipocortin (lipocortin being another name
       for lipomodulin).
DETD   . . .  (IgE-PF) to producing IgE binding factors having suppressive
       activity (IgE-SF), or (2) by its ability to inhibit IgE-induced
       expression of   ***Fc***  -  ***epsilon***     ***receptors***   on
       lymphocytes. Below, assays applicable to rodent GIFs are described. Some
       routine experimentation may be required to develop analogous assays.  .
       .  23B6 cells (deposited with the American Type Culture Collection,
       Rockville, MD, under accession number HB8521) have been shown to switch
         ***human***   T cell hybridoma 166A2 (described by Huff and Ishizaka in
       Proc. Natl. Acad. Sci., Vol. 81, pg. 1514 [1984]) from.  .  .
DETD   . . .  factors in either the effluent or eluate fractions are measured
       by their ability to inhibit rosette formation between cells having
         ***Fc***  -  ***epsilon***     ***receptors***   and IgE-coated
       erythrocytes. Red cells coated with   ***human***   serum albumin (HSA)
       are used to indicate the level of nonspecific rosettes. IgE-coated
       erythrocytes are prepared by the method disclosed.  .  .  Plainview,
       N.Y.), and formaldehyde, and the fixed cells are kept in
       phosphate-buffered saline (PBS), pH7.2. Sensitization of fixed
       erythrocytes with   ***human***   IgE, rat IgE, or   ***human***   serum
       albumin (HSA, 2.times.crystallized, Nutritional Biochemicals, Cleveland,
       Ohio) is carried out in 0.1M acetate buffer, pH 5.0. A 4% suspension.  .
       .  room temperature. An optimal concentration of each protein for
       sensitization is determined by preliminary experiments. Preferably,
       about 0.2 mg/ml of   ***human***   IgE, 0.25 mg/ml of rat IgE, and 0.25
       mg/ml of HSA is employed to sensitize the cells. A 1% suspension.  .  .
DETD   . . .  rat (e.g. Lewis strain, available from Microbiological
       Associates) infected with the nematode, Nippostrongylus brasiliensis
       (Nb) can be the source of   ***Fc***  -epsilon positive cells for the
       assay. For example, a rat can be infected with 2800-3000 larvae of Nb
       via a subcutaneous.  .  .


=> s l10 and ((carbohydrate(4w) (detectable marker))
UNMATCHED LEFT PARENTHESIS 'AND ((CARBOHYDR'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s l10 and ((carbohydrate(4w)detectable marker))
L11          0 L10 AND ((CARBOHYDRATE(4W) DETECTABLE MARKER))

=> s l10 and detectable marker
L12         24 L10 AND DETECTABLE MARKER

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13          24 DUP REM L12 (0 DUPLICATES REMOVED)

=> d 24 bib ab kwic

L13  ANSWER 24 OF 24  USPATFULL on STN
AN     1999:102683  USPATFULL
TI     Method to detect IgE
IN     Frank, Glenn R., Wellington, CO, United States
       Porter, James P., Fort Collins, CO, United States
       Rushlow, Keith E., Fort Collins, CO, United States
       Wassom, Donald L., Fort Collins, CO, United States
PA     Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI     US 5945294              19990831
```

AI US 1996-756387 19961126 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James; Assistant Examiner: Swartz, Rodney P.
LREP Heska Corporation
CLMN Number of Claims: 77
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 2155
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.
AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention. . .
SUMM Until the discovery of the present invention, detection of IgE in samples obtained from non- ***human*** animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to. . . with other antibody idotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) molecule to detect the presence of IgE in a putative IgE-containing composition. A ***Fc*** .sub..epsilon. R molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a ***Fc*** .sub..epsilon. R molecule can bind to an IgE with more specificity (i.e., less isotype cross-reactivity) and more sensitivity (i.e., affinity) than. . .
SUMM Lowenthal et al., 1993, Annals of Allergy 71:481-484, disclose that dog serum can transfer cutaneous reactivity to a ***human*** . While it is possible that Lowenthal et al. properly teach the binding of ***human*** ***Fc*** .sub..epsilon. R to canine IgE. Lowenthal et al., however, do not provide data defining the particular cellular proteins responsible for the. . . by Lowenthal et al. is merely an interpretation. In addition, Lowenthal et al. do not teach the use of purified ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE. The subunits of ***human*** ***Fc*** .sub..epsilon. R have been known as early as 1988 and have never been used to detect canine, feline or equine IgE. Indeed, U.S. Pat. No. 4,962,035, to Leder et al., issued Oct. 9, 1990, discloses ***human*** ***Fc*** .sub..epsilon. R but does not disclose the use of such a ***human*** ***Fc*** .sub..epsilon. R to detect ***human*** or non- ***human*** IgE. The use of purified ***human*** ***Fc*** .sub..epsilon. R avoids complications presented by use of ***Fc*** .sub..epsilon. R bound to a cell, such as non-specific binding of the ***Fc*** .sub..epsilon. R-bearing cell due to additional molecules present on the cell membrane. That purified ***human*** ***Fc*** .sub..epsilon. R detects non- ***human*** IgE is unexpected because inter-species binding between a ***Fc*** .sub..epsilon. R and an IgE is not predictable. For example, ***human*** ***Fc*** .sub..epsilon. R binds to rat IgE but rat ***Fc*** .sub..epsilon. R does not bind to ***human*** IgE.
SUMM The high affinity ***Fc*** .sub..epsilon. R consists of three protein chains, alpha, beta and gamma. Prior investigators have disclosed the nucleic acid sequence for: the. . .
SUMM Thus, methods and kits are needed in the art that will provide specific detection of non- ***human*** IgE.
SUMM . . . that detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex, wherein the IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon. R molecule:IgE complex, the presence of the ***Fc*** .sub..epsilon. R molecule:IgE complex indicating the presence of IgE. A preferred ***Fc*** .sub..epsilon. R molecule in which a carbohydrate group of the

Fc .sub..epsilon. R molecule is conjugated to biotin.

SUMM . . . conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell includes: a recombinant cell expressing a ***human*** ***Fc*** .sub..epsilon. R molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE including canine IgE, feline IgE. . .

SUMM . . . binding to the substrate; and (c) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with a ***Fc*** .sub..epsilon. R molecule. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva. . .

SUMM . . . includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting an IgE including canine IgE, feline IgE and equine IgE. Another embodiment is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule. Another embodiment is a kit for detecting flea allergy dermatitis comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a flea allergen.

SUMM Another embodiment of the present invention is an isolated ***human*** ***Fc*** receptor (***Fc*** .sub..epsilon. R) alpha chain protein, in which a carbohydrate group of the ***Fc*** .sub..epsilon. R alpha chain protein is conjugated to biotin. A preferred ***Fc*** .sub..epsilon. R alpha chain protein comprises PhFc.sub..epsilon. R.alpha..sub.172 -BIOT.

DRWD FIG. 1 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies.

DRWD FIG. 2 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect plant allergen-specific canine IgE antibodies.

DRWD FIG. 3 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect ***human*** or canine IgE antibodies.

DRWD FIG. 4 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea allergen-specific canine IgE antibodies.

DRWD FIG. 5 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea allergen-specific and heartworm antigen-specific canine IgE antibodies.

DRWD FIG. 6 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea saliva-specific canine IgE antibodies.

DRWD FIG. 7 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect heartworm antigen-specific feline IgE antibodies.

DRWD FIG. 8 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect heartworm antigen-specific feline IgE antibodies.

DRWD FIG. 9 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect antigen-specific equine IgE antibodies.

DRWD FIG. 10 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies in sera from heartworm-infected dogs.

DRWD FIG. 11 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies in sera from flea saliva sensitized dogs.

DETD The present invention relates to the discovery that purified high affinity ***human*** ***Fc*** ***epsilon*** ***receptor*** (i.e., ***Fc*** .sub..epsilon. RI; referred to herein as ***Fc*** .sub..epsilon. R) can be used in certain non- ***human*** (i.e., canine, feline or equine) epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of ***human*** ***Fc*** .sub..epsilon. R to detect non- ***human*** IgE is unexpected because canine, feline and equine immune systems are different from the ***human*** immune system, as well as from each other (i.e., molecules important to the

immune system usually are species specific).

DETD One embodiment of the present invention is a method to detect a non- ***human*** IgE using an isolated ***human*** ***Fc*** .sub..epsilon. R molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more. . .

DETD According to the present invention, an isolated, or biologically pure, ***Fc*** .sub..epsilon. R molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention can be obtained from its natural source (e.g., from a ***human*** mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

DETD A ***Fc*** .sub..epsilon. R molecule (also referred to herein as ***Fc*** .sub..epsilon. R or ***Fc*** .sub..epsilon. R protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A ***Fc*** .sub..epsilon. R molecule of the present invention can comprise a complete ***Fc*** .sub..epsilon. R (i.e., alpha, beta and gamma ***Fc*** .sub..epsilon. R chains), an alpha ***Fc*** .sub..epsilon. R chain (also referred to herein as ***Fc*** .sub..epsilon. R a chain) or portions thereof. Preferably, a ***Fc*** .sub..epsilon. R molecule comprises at least a portion of a ***Fc*** .sub..epsilon. R a chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. Preferably, a ***Fc*** .sub..epsilon. R molecule of the present invention binds to IgE with an affinity of about K.sub.A .apprxeq.10.sup.8, more preferably with an. . .

DETD An isolated ***Fc*** .sub..epsilon. R molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the ***Fc*** .sub..epsilon. R molecule's ability to form an immunocomplex with an IgE. Examples of ***Fc*** .sub..epsilon. R homologs include ***Fc*** .sub..epsilon. R proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide),. . .

DETD ***Fc*** .sub..epsilon. R homologs can be the result of natural allelic variation or natural mutation. ***Fc*** .sub..epsilon. R homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

DETD According to the present invention, a ***human*** ***Fc*** .sub..epsilon. R .alpha. chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein represented herein as SEQ ID NO: 1, the portion at least encoding the IgE binding site of the ***Fc*** .sub..epsilon. R .alpha. chain protein. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO: 1 and. . . determined by one skilled in the art and is shown herein as SEQ ID NO:3) is referred to herein as ***Fc*** .sub..epsilon. R nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198. Translation of SEQ ID NO: 1 suggests that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198 encodes a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.257, represented by SEQ ID NO:2,. . . skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a ***Fc*** .sub..epsilon. R .alpha. chain protein with increased solubility and/or a truncated protein (e.g., a peptide) capable of detecting IgE, e.g., PhFc.sub..epsilon. R.alpha..sub.197 and PhFc.sub..epsilon. R.alpha..sub.172. Preferred ***Fc*** .sub..epsilon. R molecules include PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc!sub..epsilon. R.alpha..sub.172. Preferred nucleic acid molecules to encode a ***Fc*** .sub..epsilon. R molecules include nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon. R.alpha..sub.612, nhFc.sub..epsilon. R.alpha..sub.591, nhFc.sub..epsilon. R.alpha..sub.699 and/or nhFc.sub..epsilon. R.alpha..sub.516.

DETD Isolated ***Fc*** .sub..epsilon. R molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under. . . Suitable and preferred nucleic acid molecules with

which to transform a cell are as disclosed herein for suitable and preferred ***Fc*** .sub..epsilon. R nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention. . .

DETD . . . one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a ***Fc*** .sub..epsilon. R molecule protein of the present invention or can be capable of producing such proteins after being transformed with at. . .

DETD . . . transformed, examples of which are disclosed herein. A particularly preferred recombinant molecule includes pVL-nhFc.sub..epsilon. R.alpha..sub.612. Details regarding the production of ***Fc*** .sub..epsilon. R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cell of the present invention includes Trichoplusia. . .

DETD A ***Fc*** .sub..epsilon. R molecule of the present invention can include chimeric molecules comprising a portion of a ***Fc*** .sub..epsilon. R molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon. R portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon. R molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of. . .

DETD A ***Fc*** .sub..epsilon. R molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon. R formulation. For example, a ***Fc*** .sub..epsilon. R can be combined with a buffer in which the ***Fc*** .sub..epsilon. R is solubilized, and/or a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a ***Fc*** .sub..epsilon. R can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be in mixed with ***Fc*** .sub..epsilon. R or conjugated (i.e., attached) to ***Fc*** .sub..epsilon. R in such a manner as to not substantially interfere with the ability of the ***Fc*** .sub..epsilon. R to selectively bind to IgE.

DETD A ***Fc*** .sub..epsilon. R of the present invention can be bound to the surface of a cell expressing the ***Fc*** .sub..epsilon. R. A preferred ***Fc*** .sub..epsilon. R-bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon. R alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid. . .

DETD In addition, a ***Fc*** .sub..epsilon. R formulation of the present invention can include not only a ***Fc*** .sub..epsilon. R but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

DETD The present invention also includes ***human*** ***Fc*** .sub..epsilon. R mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a ***Fc*** .sub..epsilon. R molecule to bind to IgE. A mimetope can be a peptide that has been modified to decrease its susceptibility. . . by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of ***Fc*** .sub..epsilon. R mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins. . .

DETD One embodiment of the present invention is a method to detect non-***human*** IgE which includes the steps of: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon. R molecule:IgE complex; and (b) detecting levels of IgE by detecting said ***Fc*** .sub..epsilon. R molecule:IgE complex. Presence of such a ***Fc*** .sub..epsilon. R molecule:IgE complex indicates that the animal is producing IgE. Preferred non-***human*** IgE to detect using a ***human*** ***Fc*** .sub..epsilon. R molecule include canine IgE, feline IgE and equine IgE. The present

method can further include the step of determining whether an IgE complexed with a ***Fc*** .sub..epsilon. R molecule is heat labile. Methods to determine heat lability of IgE are disclosed in the Examples section. Preferably, an. . . certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a ***human*** ***Fc*** .sub..epsilon. R suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a ***Fc*** .sub..epsilon. R molecule of the present invention may be useful for detecting molecules bound by the ***Fc*** .sub..epsilon. R molecule but not identical to a known IgE.

DETD As used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a ***human*** ***Fc*** .sub..epsilon. R molecule. Formation of a complex between a ***Fc*** .sub..epsilon. R and an IgE refers to the ability of the ***Fc*** .sub..epsilon. R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a ***Fc*** .sub..epsilon. R of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a ***Fc*** .sub..epsilon. R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

DETD . . . complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between ***Fc*** .sub..epsilon. R and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

DETD . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the ***Fc*** .sub..epsilon. R or to a reagent that selectively binds to the ***Fc*** .sub..epsilon. R or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin). Preferably, biotin is conjugated to an alpha chain of a ***Fc*** .sub..epsilon. R. Preferably a carbohydrate group of the ***Fc*** .sub..epsilon. R alpha chain is conjugated to biotin. A preferred ***Fc*** .sub..epsilon. R molecule conjugated to biotin comprises PhFc.sub..epsilon. R.alpha..sub.172 -BIOT (the production of which is described in the Examples section).

DETD In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon. R molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a ***Fc*** .sub..epsilon. R molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A ***detectable*** ***marker*** is conjugated to a ***Fc*** .sub..epsilon. R molecule or a reagent in such a manner as not to block the ability of the ***Fc*** .sub..epsilon. R or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a ***Fc*** .sub..epsilon. R is conjugated to biotin.

DETD In another embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon. R molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the ***Fc*** .sub..epsilon. R molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a ***Fc*** .sub..epsilon. R molecule, an antigen, an antibody and a lectin, depending upon which portion of the ***Fc*** .sub..epsilon. R molecule:IgE complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- ***Fc*** .sub..epsilon. R antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a ***Fc*** .sub..epsilon. R molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a

detectable ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

DETD In one preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a ***Fc*** .sub..epsilon. R molecule of the present invention. Examples of such a reagent includes, but are not limited to, an antibody that selectively binds to a ***Fc*** .sub..epsilon. R molecule (referred to herein as an anti- ***Fc*** .sub..epsilon. R antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon. R molecule. ***Fc*** .sub..epsilon. R molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

DETD In another preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an ***Fc*** receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9, and CMI antibody #9,. . .

DETD . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

DETD A preferred immunoabsorbent assay method includes a step of either: (a) binding an ***Fc*** .sub..epsilon. R molecule to a substrate prior to contacting a ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a ***Fc*** .sub..epsilon. R molecule-coated substrate; or (b) binding a putative IgE-containing composition to a substrate prior to contacting a ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate includes of a non-coated substrate, a ***Fc*** .sub..epsilon. R molecule-coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

DETD . . . upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a ***Fc*** .sub..epsilon. R molecule of the present invention is used as a capture molecule when the ***Fc*** .sub..epsilon. R molecule is bound to a substrate. Alternatively, a ***Fc*** .sub..epsilon. R molecule is used as an indicator molecule when the ***Fc*** .sub..epsilon. R molecule is not bound to a substrate. Suitable molecule for use as capture molecules or indicator molecules include, but are not limited to, a ***Fc*** .sub..epsilon. R molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

DETD . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

DETD . . . molecule that can selectively bind to an IgE bound to the antigen, the indicator molecule can be conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a ***Fc*** .sub..epsilon. R molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

DETD In one embodiment, a ***Fc*** .sub..epsilon. R molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well. . . A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for ***Fc*** .sub..epsilon. R molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain ***Fc***

.sub..epsilon. R molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the ***Fc*** .sub..epsilon. R is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the ***Fc*** .sub..epsilon. R molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

DETD . . . material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon. R molecule and the anti-IgE antibody:IgE complex. Preferably, the ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . .

DETD . . . Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon. R molecule and the IgE. Preferably, the ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . .

DETD . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a calorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a ***Fc*** .sub..epsilon. R molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent. . .

DETD . . . apparatus used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a ***Fc*** .sub..epsilon. R molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a. . .

DETD . . . assay in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a ***Fc*** .sub..epsilon. R molecule of the present invention and an isolated IgE known to bind to the ***Fc*** .sub..epsilon. R molecule. The absence of binding of the ***Fc*** .sub..epsilon. R molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

DETD . . . detect IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting an IgE including canine IgE, feline IgE and/or equine IgE. Suitable and preferred ***Fc*** .sub..epsilon. R molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the ***Fc*** .sub..epsilon. R molecule or to an IgE. A preferred kit of the present invention further comprises a detection means including one. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon. R molecule (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

DETD . . . present invention is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that. . .

DETD . . . such as cod, halibut or and tuna, egg, milk, Brewer's yeast,

whole wheat, corn, soybean, cheese and rice, and a ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

DETD This example describes the construction of a recombinant baculovirus expressing a truncated portion of the .alpha.-chain of the ***human*** ***Fc*** .sub..epsilon. receptor.

DETD Recombinant molecule pVL-nhFc.sub..epsilon. R.alpha..sub.612, containing a nucleic acid molecule encoding the extracellular domain of the ***Fc*** .sub..epsilon. R .alpha. chain, operatively linked to baculovirus polyhedron transcription control sequences was produced in the following manner. A cDNA clone encoding the full-length alpha chain (.alpha. chain) of the ***human*** ***Fc*** .sub..epsilon. receptor was obtained from Dr. Jean-Pierre Kinet (Harvard University, Cambridge, Mass.). The cDNA clone included an about 1198 nucleotide insert,. . . SEQ ID NO: 1. Translation of SEQ ID NO: 1 indicates that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198 encodes a full-length ***human*** ***Fc*** .sub..epsilon. receptor .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.257, having amino acid sequence SEQ. . . 1. The complement of SEQ ID NO: 1 is represented herein by SEQ ID NO:3. The proposed mature protein (i.e., ***Fc*** .sub..epsilon. R.alpha. chain from which the signal sequence has been cleaved), denoted herein as PhFc.sub..epsilon. R.alpha..sub.232, contains about 232 amino acids. . .

DETD To produce a secreted form of the extracellular domain of the ***Fc*** .sub..epsilon. R .alpha. chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the ***Fc*** .sub..epsilon. R .alpha. chain encoded by nhFc.sub..epsilon. R.alpha..sub.1198 were removed as follows. A ***Fc*** .sub..epsilon. R .alpha. chain extracellular domain nucleic acid molecule-containing fragment of about 612 nucleotides was PCR amplified from nhFc.sub..epsilon. R.alpha..sub.1198 using. . . produce nhFc.sub..epsilon. R.alpha..sub.612. Nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 contained an about 591 nucleotide fragment encoding the extracellular domain of the ***human*** ***Fc*** .sub..epsilon. R .alpha. chain, extending from about nucleotide 107 to about nucleotide 697 of SEQ ID NO 1, denoted herein as. . . denoted SEQ ID NO: 10. Translation of SEQ ID NO: 10 indicates that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 encodes a ***Fc*** .sub..epsilon. R protein of about 197 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.197, having amino acid sequence SEQ ID NO: 11. Nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 encodes a secretable form of the ***human*** ***Fc*** .sub..epsilon. R .alpha. chain which does not possess a leader sequence, which is denoted herein as PhFc.sub..epsilon. R.alpha..sub.172 having amino acid. . .

DETD This example describes the biotinylation of a recombinant ***human*** ***Fc*** .sub..epsilon. R alpha chain protein.

DETD The results shown in FIG. 1 indicate that the alpha chain of ***human*** ***FcR*** (solid squares) detects the presence of canine IgE in a solid-phase assay in a similar manner as the control antibody. . .

DETD The results shown in FIG. 2 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon. R detects the presence of canine IgE antibodies that bind specifically to a common grass allergen or to a common. . .

DETD . . . in FIG. 3 indicate that canine IgE from a variety of dog sera are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R in a manner similar to using an antibody that binds specifically to canine IgE. The absence of detectable amounts. . .

DETD . . . 4 indicate that canine IgE that binds specifically to a flea saliva antigen is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD . . . IgE from dogs allergic to flea saliva and from dogs infected with heartworm are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the anti-IgE antibody and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody and not another isotype.

DETD . . . indicate that canine IgE that binds specifically to flea saliva, contained in serum, is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of

colorimetric signal in samples of heat inactivated serum indicates that antibody bound to the flea saliva protein and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody.

DETD . . . feline IgE that binds specifically to crude homogenate of heartworm or Di33 protein is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD . . . feline IgE from heartworm-infected cats that specifically binds to the heartworm antigen Di33 is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the Di33 protein and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody.

DETD . . . be allergic to certain allergens specifically binds to certain plant and mite allergens is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD This example describes detection of canine IgE in a solid-phase ELISA using basophilic cells transfected with ***human*** ***Fc*** .sub..epsilon. R alpha chain.

DETD Rat basophilic leukemia (RBL) cells transfected with a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon. R alpha chain (referred to herein as RBL-hFc.sub..epsilon. R cells; described in Miller et al., Science 244:334-337, 1989) were used. . .

DETD The results shown in FIG. 10 indicate that canine IgE from heartworm-infected dogs (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon. R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of calorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon. R alpha chain on the RBL-h ***Fc*** .sub..epsilon. R cells is an epsilon isotype antibody. Similarly, the results shown in FIG. 11 indicate that canine IgE from dogs sensitized with flea saliva (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon. R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon. R alpha chain on the RBL-h ***Fc*** .sub..epsilon. R cells is an epsilon isotype antibody.

CLM What is claimed is:

1. A method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule comprising at least a portion of a ***human*** ***Fc*** .sub..epsilon. R alpha chain that binds to IgE with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex, wherein said IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting said ***Fc*** .sub..epsilon. R molecule:IgE complex, the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex indicating the presence of IgE.

2. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172.

. . .

3. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon.. . .

4. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising. . .

5. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

6. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .

7. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, biotin-related compounds, avidin, avidin-related compounds and a peroxidase.

. . .

8. The method of claim 1, wherein a carbohydrate group of said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

13. The method of claim 1 further comprising the step selected from the group consisting of binding said ***Fc*** .sub..epsilon. R molecule to a substrate prior to performing step (a) to form a ***Fc*** .sub..epsilon. R molecule-coated substrate and binding said putative IgE-containing composition to a substrate prior to performing step (a) to form a putative IgE-containing composition-coated substrate, wherein said substrate is selected from the group consisting of a non-coated substrate, a ***Fc*** .sub..epsilon. R molecule-coated substrate, an antigen-coated substrate and an anti-IgE antibody-coated substrate.

21. The method of claim 1, wherein said step of detecting comprises: (a) contacting said ***Fc*** .sub..epsilon. R molecule:IgE complex with an indicator molecule that binds selectively to said ***Fc*** .sub..epsilon. R molecule:IgE complex; (b) removing substantially all of said indicator molecule that does not selectively bind to ***Fc*** .sub..epsilon. R molecule:IgE complex; and (c) detecting said indicator molecule, wherein presence of said indicator molecule is indicative of the presence. . .

. . . 22. The method of claim 21, wherein said indicator molecule comprises a compound selected from the group consisting of a ***Fc*** .sub..epsilon. R molecule, an antigen, an antibody and a lectin.

23. The method of claim 1, said method comprising the steps of: (a) immobilizing said ***Fc*** .sub..epsilon. R molecule on a substrate; (b) contacting said ***Fc*** .sub..epsilon. R molecule with said putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon. R molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon. R molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex.

24. The method of claim 23, wherein the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon. R molecule:IgE complex with a compound selected from the group consisting of an antigen and an antibody that binds selectively. . .

25. The method of claim 24, wherein said compound comprises a ***detectable*** ***marker*** .

26. The method of claim 1, said method comprising the steps of: (a) immobilizing a desired antigen on a substrate;. . . binding to said substrate; and (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with said ***Fc*** .sub..epsilon. R molecule.

27. The method of claim 26, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, avidin, a peroxidase and other members of the. .

. . . binding to said substrate; and (d) detecting the presence of said antibody:IgE complex by contacting said antibody:IgE complex with said ***Fc*** .sub..epsilon. R molecule.

29. The method of claim 28, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

. . . method comprising the steps of: (a) immobilizing said putative IgE-containing composition on a substrate; (b) contacting said

composition with said ***Fc*** .sub..epsilon. R molecule under conditions suitable for formation of an ***Fc*** .sub..epsilon. R molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon. R molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex.

31. The method of claim 30, wherein the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon. R molecule:IgE complex with an indicator molecule selected from the group consisting of an antibody, an antigen and a lectin.

. . .

32. The method of claim 30, wherein said ***Fc*** .sub..epsilon. R molecule comprises a ***detectable*** ***marker*** .

35. A kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor molecule comprising at least a portion of a ***human*** ***Fc*** .sub..epsilon. R alpha chain that binds to IgE and a means for detecting an IgE selected from the group consisting of.

. .

38. The kit of claim 35, wherein said detection means detects said ***Fc*** .sub..epsilon. R molecule.

39. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

46. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172.

. . .

47. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon.. . .

48. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising. . .

49. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

50. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .

51. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

52. The kit of claim 35, wherein a carbohydrate group of said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

. . . said labeling reagent is impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising said ***Fc*** .sub..epsilon. R molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to. . .

65. A general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor molecule comprising at least a portion of a ***human*** ***Fc*** .sub..epsilon. R alpha chain that binds to IgE.

71. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172.

. . .
72. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon.. . .
73. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising. . .
74. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

75. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a ligand.
. . .
76. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .
77. The kit of claim 65, wherein a carbohydrate group of said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

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YOU HAVE REQUESTED DATA FROM 24 ANSWERS - CONTINUE? Y/(N):y

L13 ANSWER 1 OF 24 USPATFULL on STN
AN 2004:273712 USPATFULL
TI Method to detect IgE
IN Frank, Glenn R., Wellington, CO, UNITED STATES
Porter, James P., Fort Collins, CO, UNITED STATES
Rushlow, Keith E., Fort Collins, CO, UNITED STATES
Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI US 2004214209 A1 20041028
AI US 2004-763400 A1 20040123 (10)
RLI Division of Ser. No. US 2001-944277, filed on 30 Aug 2001, GRANTED, Pat. No. US 6682894 Division of Ser. No. US 1999-285873, filed on 31 Mar 1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 10
ECL Exemplary Claim: 106
DRWN 11 Drawing Page(s)
LN.CNT 1870
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) to detect IgE antibodies in a biological sample from a cat dog, horse or mouse. The present invention also relates to kits to perform such methods.
AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) to detect IgE antibodies in a biological sample from a cat dog, horse or mouse. The present invention also relates. . .
SUMM [0003] Until the discovery of the present invention, detection of IgE in samples obtained from non- ***human*** animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to. . . with other antibody idiotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) molecule to detect the presence of IgE in a putative IgE-containing composition. A ***Fc*** .sub..epsilon.R molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a ***Fc*** .sub..epsilon.R molecule can bind to an IgE with more specificity (i.e., less idiotype cross-reactivity) and more

sensitivity (i.e., affinity) than anti-IgE. . .

SUMM [0004] Lowenthal et al., 1993, Annals of Allergy 71:481-484, dog serum can transfer cutaneous reactivity to a ***human*** . While it is possible that Lowenthal et al. properly teach the binding of ***human*** ***Fc*** .sub..epsilon.R to canine IgE. Lowenthal et al., however, do not provide data defining the particular cellular proteins responsible for the transfer. . . by Lowenthal et al. is merely an interpretation. In addition, Lowenthal et al. do not teach the use of purified ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE. The subunits of ***human*** ***Fc*** .sub..epsilon.R have been known as early as 1988 and have never been used to detect canine, feline or equine IgE. Indeed, U.S. Pat. No. 4,962,035, to Leder et al., issued Oct. 9, 1990, discloses ***human*** ***Fc*** .sub..epsilon.R but does not disclose the use of such a ***human*** ***Fc*** .sub..epsilon.R to detect ***human*** or non- ***human*** IgE. The use of purified ***human*** ***Fc*** .sub..epsilon.R avoids complications presented by use of ***Fc*** .sub..epsilon.R bound to a cell, such as non-specific binding of the ***Fc*** .sub..epsilon.R-bearing cell due to additional molecules present on the cell membrane. That purified ***human*** ***Fc*** .sub..epsilon.R detects non- ***human*** IgE is unexpected because inter-species binding between a ***Fc*** .sub..epsilon.R and an IgE is not predictable. For example, ***human*** ***Fc*** .sub..epsilon.R binds to rat IgE but rat ***Fc*** .sub..epsilon.R does not bind to ***human*** IgE.

SUMM [0005] The high affinity ***Fc*** .sub..epsilon.R consists of three protein chains, alpha, beta and gamma. Prior investigators have disclosed the nucleic acid sequence for: the alpha. . .

SUMM [0006] Thus, methods and kits are needed in the art that will provide specific detection of non- ***human*** IgE.

SUMM . . . that detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex, wherein the IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon.R molecule:IgE complex, the presence of the ***Fc*** .sub..epsilon.R molecule:IgE complex indicating the presence of IgE. A preferred ***Fc*** .sub..epsilon.R molecule in which a carbohydrate group of the ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

SUMM . . . conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell includes: a recombinant cell expressing a ***human*** ***Fc*** .sub..epsilon.R molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE including canine IgE, feline IgE and. . .

SUMM . . . binding to the substrate; and (c) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with a ***Fc*** .sub..epsilon.R molecule. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva proteins.

SUMM . . . includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule and a means for detecting an IgE including canine IgE, feline IgE and equine IgE. Another embodiment is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule. Another embodiment is a kit for detecting flea allergy dermatitis comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule and a flea allergen.

SUMM [0011] Another embodiment of the present invention is an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) alpha chain protein, in which a carbohydrate group of the ***Fc*** .sub..epsilon.R alpha chain protein is conjugated to biotin. A preferred ***Fc*** .sub..epsilon.R alpha chain protein comprises PhFc.sub..epsilon.R.alpha..sub.172-BIOT.

DRWD [0012] FIG. 1 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE antibodies.

DRWD [0013] FIG. 2 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect plant allergen-specific canine IgE antibodies.

DRWD [0014] FIG. 3 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect ***human*** or canine IgE antibodies.

DRWD [0015] FIG. 4 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect flea allergen-specific canine IgE antibodies.

DRWD [0016] FIG. 5 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect flea allergen-specific and heartworm antigen-specific canine IgE antibodies.

DRWD [0017] FIG. 6 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect flea saliva-specific canine IgE antibodies.

DRWD [0018] FIG. 7 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect heartworm antigen-specific feline IgE antibodies.

DRWD [0019] FIG. 8 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect heartworm antigen-specific feline IgE antibodies.

DRWD [0020] FIG. 9 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect antigen-specific equine IgE antibodies.

DRWD [0021] FIG. 10 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE antibodies in sera from heartworm-infected dogs.

DRWD [0022] FIG. 11 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE antibodies in sera from flea saliva sensitized dogs.

DETD [0023] The present invention relates to the discovery that purified high affinity ***human*** ***Fc*** ***epsilon*** ***receptor*** (i.e., ***Fc*** .sub..epsilon.RI; referred to herein as ***Fc*** .sub..epsilon.R) can be used in certain non- ***human*** (i.e., canine, feline or equine) epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of ***human*** ***Fc*** .sub..epsilon.R to detect non- ***human*** IgE is unexpected because canine, feline and equine immune systems are different from the ***human*** immune system, as well as from each other (i.e., molecules important to the immune system usually are species specific).

DETD [0024] One embodiment of the present invention is a method to detect a non- ***human*** IgE using an isolated ***human*** ***Fc*** .sub..epsilon.R molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more of. . .

DETD [0025] According to the present invention, an isolated, or biologically pure, ***Fc*** .sub..epsilon.R molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated ***human*** ***Fc*** .sub..epsilon.R molecule of the present invention can be obtained from its natural source (e.g., from a ***human*** mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

DETD [0026] A ***Fc*** .sub..epsilon.R molecule (also referred to herein as ***Fc*** .sub..epsilon.R or ***Fc*** .sub..epsilon.R protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A ***Fc*** .sub..epsilon.R molecule of the present invention can comprise a complete ***Fc*** .sub..epsilon.R (i.e., alpha, beta and gamma ***Fc*** .sub..epsilon.R chains), an alpha ***Fc*** .sub..epsilon.R chain (also referred to herein as ***Fc*** .sub..epsilon.R .alpha. chain) or portions thereof. Preferably, a ***Fc*** .sub..epsilon.R molecule comprises at least a portion of a ***Fc*** .sub..epsilon.R .alpha. chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. Preferably, a ***Fc*** .sub..epsilon.R molecule of the present invention binds to IgE with an affinity of about K.sub.A.apprxeq.10.sup.8, more preferably with an affinity of. . .

DETD [0027] An isolated ***Fc*** .sub..epsilon.R molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the ***Fc*** .sub..epsilon.R molecule's ability to form an

immunocomplex with an IgE. Examples of ***Fc*** .sub..epsilon.R homologs include ***Fc*** .sub..epsilon.R proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted,. . .

DETD [0028] ***Fc*** .sub..epsilon.R homologs can be the result of natural allelic variation or natural mutation. ***Fc*** .sub..epsilon.R homologs of the present invention can also be produced using techniques known in the art including, but not limited to,. . .

DETD [0029] According to the present invention, a ***human*** ***Fc*** .sub..epsilon.R .alpha. chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length ***Fc*** .sub..epsilon.R .alpha. chain protein represented herein as SEQ ID NO:1, the portion at least encoding the IgE binding site of the ***Fc*** .sub..epsilon.R .alpha. chain protein. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO:1 and the complementary. . . determined by one skilled in the art and is shown herein as SEQ ID NO:3) is referred to herein as ***Fc*** .sub..epsilon.R nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.1198. Translation of SEQ ID NO:1 suggests that nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.1198 encodes a full-length ***Fc*** .sub..epsilon.R .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon.R.alpha..sub.257, represented by SEQ ID NO:2, assuming an. . . skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a ***Fc*** .sub..epsilon.R.alpha. chain protein with increased solubility and/or a truncated protein (e.g., a peptide) capable of detecting IgE, e.g., PhFc.sub..epsilon.R.alpha..sub.197 and PhFc.sub..epsilon.R.alpha..sub.172. Preferred ***Fc*** .sub..epsilon.R molecules include PhFc.sub..epsilon.R.alpha..sub.257, PhFc.sub..epsilon.R.alpha..sub.197, PhFc.sub..epsilon.R.alpha..sub.232 and PhFc.sub..epsilon.R.alpha..sub.172. Preferred nucleic acid molecules to encode a ***Fc*** .sub..epsilon.R molecules include nhFc.sub..epsilon.R.alpha..sub.774, nhFc.sub..epsilon.R.alpha..sub.1198, nhFc.sub..epsilon.R.alpha..sub.612, nhFc.sub..epsilon.R.alpha..sub.591, nhFc.sub..epsilon.R.alpha..sub.699 and/or nhFc.sub..epsilon.R.alpha..sub .516.

DETD [0030] Isolated ***Fc*** .sub..epsilon.R molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under conditions. . . Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred ***Fc*** .sub..epsilon.R nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention include. . .

DETD . . . one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a ***Fc*** .sub..epsilon.R molecule protein of the present invention or can be capable of producing such proteins after being transformed with at least. . .

DETD . . . be transformed, examples of which are disclosed herein. A particularly preferred recombinant molecule includes pVL-nhFc.sub..epsilon.R.alpha..sub.612. Details regarding the production of ***Fc*** .sub..epsilon.R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cell of the present invention includes Trichoplusia ni-pVL-nhFc.sub..epsilon.R.alpha..sub.612.

DETD [0033] A ***Fc*** .sub..epsilon.R molecule of the present invention can include chimeric molecules comprising a portion of a ***Fc*** .sub..epsilon.R molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon.R portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon.R molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an. . .

DETD [0034] A ***Fc*** .sub..epsilon.R molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon.R formulation. For example, a ***Fc*** .sub..epsilon.R can be combined with a buffer in which the ***Fc*** .sub..epsilon.R is solubilized, and/or a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a ***Fc*** .sub..epsilon.R can function to

selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer,. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be in mixed with ***Fc*** .sub..epsilon.R or conjugated (i.e., attached) to ***Fc*** .sub..epsilon.R in such a manner as to not substantially interfere with the ability of the ***Fc*** .sub..epsilon.R to selectively bind to IgE.

DETD [0035] A ***Fc*** .sub..epsilon.R of the present invention can be bound to the surface of a cell expressing the ***Fc*** .sub..epsilon.R. A preferred ***Fc*** .sub..epsilon.R-bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon.R alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid molecule. . .

DETD [0036] In addition, a ***Fc*** .sub..epsilon.R formulation of the present invention can include not only a ***Fc*** .sub..epsilon.R but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers to. . .

DETD [0038] The present invention also includes ***human*** ***Fc*** .sub..epsilon.R mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a ***Fc*** .sub..epsilon.R molecule to bind to IgE. A mimetope can be a peptide that has been modified to decrease its susceptibility to. . . by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of ***Fc*** .sub..epsilon.R mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins identified. . .

DETD [0039] One embodiment of the present invention is a method to detect non- ***human*** IgE which includes the steps of: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule with a putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon.R molecule:IgE complex; and (b) detecting levels of IgE by detecting said ***Fc*** .sub..epsilon.R molecule:IgE complex. Presence of such a ***Fc*** .sub..epsilon.R molecule:IgE complex indicates that the animal is producing IgE. Preferred non- ***human*** IgE to detect using a ***human*** ***Fc*** .sub..epsilon.R molecule include canine IgE, feline IgE and equine IgE. The present method can further include the step of determining whether an IgE complexed with a ***Fc*** .sub..epsilon.R molecule is heat labile. Methods to determine heat lability of IgE are disclosed in the Examples section. Preferably, an IgE. . . certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a ***human*** ***Fc*** .sub..epsilon.R suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a ***Fc*** .sub..epsilon.R molecule of the present invention may be useful for detecting molecules bound by the ***Fc*** .sub..epsilon.R molecule but not identical to a known IgE.

DETD . . . As used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a ***human*** ***Fc*** .sub..epsilon.R molecule. Formation of a complex between a ***Fc*** .sub..epsilon.R and an IgE refers to the ability of the ***Fc*** .sub..epsilon.R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a ***Fc*** .sub..epsilon.R of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a ***Fc*** .sub..epsilon.R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction. . .

DETD . . . complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between ***Fc*** .sub..epsilon.R and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in the. . .

DETD . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a

detectable ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the ***Fc*** .sub..epsilon.R or to a reagent that selectively binds to the ***Fc*** .sub..epsilon.R or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable markers. . . biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin). Preferably, biotin is conjugated to an alpha chain of a ***Fc*** .sub..epsilon.R. Preferably a carbohydrate group of the ***Fc*** .sub..epsilon.R alpha chain is conjugated to biotin. A preferred ***Fc*** .sub..epsilon.R molecule conjugated to biotin comprises PhFc.sub..epsilon.R.alpha..sub.172-BIOT (the production of which is described in the Examples section).

DETD [0046] In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon.R molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a ***Fc*** .sub..epsilon.R molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A ***detectable*** ***marker*** is conjugated to a ***Fc*** .sub..epsilon.R molecule or a reagent in such a manner as not to block the ability of the ***Fc*** .sub..epsilon.R or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a ***Fc*** .sub..epsilon.R is conjugated to biotin.

DETD [0047] In another embodiment, a ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon.R molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the ***Fc*** .sub..epsilon.R molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a ***Fc*** .sub..epsilon.R molecule, an antigen, an antibody and a lectin, depending upon which portion of the ***Fc*** .sub..epsilon.R molecule:IgE complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- ***Fc*** .sub..epsilon.R antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a ***Fc*** .sub..epsilon.R molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

DETD [0048] In one preferred embodiment, a ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a ***Fc*** .sub..epsilon.R molecule of the present invention. Examples of such a reagent includes, but are not limited to, an antibody that selectively binds to a ***Fc*** .sub..epsilon.R molecule (referred to herein as an anti- ***Fc*** .sub..epsilon.R antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon.R molecule. ***Fc*** .sub..epsilon.R molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

DETD [0049] In another preferred embodiment, a ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred to. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an ***Fc*** receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9, and CMI antibody #9,. . .

DETD . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

DETD [0052] A preferred immunoabsorbent assay method includes a step of either: (a) binding an ***Fc*** .sub..epsilon.R molecule to a substrate prior to contacting a ***Fc*** .sub..epsilon.R molecule with a putative IgE-containing composition to form a ***Fc*** .sub..epsilon.R molecule-coated substrate; or (b) binding a putative

IgE-containing composition to a substrate prior to contacting a ***Fc*** .sub..epsilon.R molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate includes of a non-coated substrate, a ***Fc*** .sub..epsilon.R molecule-coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

DETD . . . upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a ***Fc*** .sub..epsilon.R molecule of the present invention is used as a capture molecule when the ***Fc*** .sub..epsilon.R molecule is bound to a substrate. Alternatively, a ***Fc*** .sub..epsilon.R molecule is used as an indicator molecule when the ***Fc*** .sub..epsilon.R molecule is not bound to a substrate. Suitable molecule for use as capture molecules or indicator molecules include, but are not limited to, a ***Fc*** .sub..epsilon.R molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

DETD . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

DETD . . . molecule that can selectively bind to an IgE bound to the antigen, the indicator molecule can be conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a ***Fc*** .sub..epsilon.R molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

DETD [0056] In one embodiment, a ***Fc*** .sub..epsilon.R molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or. . . A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for ***Fc*** .sub..epsilon.R molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the ***Fc*** .sub..epsilon.R is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the ***Fc*** .sub..epsilon.R molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a calorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

DETD . . . material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A ***Fc*** .sub..epsilon.R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon.R molecule and the anti-IgE antibody:IgE complex. Preferably, the ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon.R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. .

DETD . . . Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A ***Fc*** .sub..epsilon.R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon.R molecule and the IgE. Preferably, the ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon.R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

DETD . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the

labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a ***Fc*** .sub..epsilon.R molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is. . .

DETD . . . apparatus used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a ***Fc*** .sub..epsilon.R molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture. . .

DETD . . . assay in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a ***Fc*** .sub..epsilon.R molecule of the present invention and an isolated IgE known to bind to the ***Fc*** .sub..epsilon.R molecule. The absence of binding of the ***Fc*** .sub..epsilon.R molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

DETD . . . detect IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule and a means for detecting an IgE including canine IgE, feline IgE and/or equine IgE. Suitable and preferred ***Fc*** .sub..epsilon.R molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the ***Fc*** .sub..epsilon.R molecule or to an IgE. A preferred kit of the present invention further comprises a detection means including one or. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon.R molecule (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

DETD . . . present invention is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon.R molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are. . .

DETD . . . such as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and rice, and a ***human*** ***Fc*** .sub..epsilon.R molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

DETD [0070] This example describes the construction of a recombinant baculovirus expressing a truncated portion of the .alpha.-chain of the ***human*** ***Fc*** .sub..epsilon. receptor.

DETD [0071] Recombinant molecule pVL-nhFc.sub..epsilon.R.alpha..sub.612, containing a nucleic acid molecule encoding the extracellular domain of the ***Fc*** .sub..epsilon.R .alpha. chain, operatively linked to baculovirus polyhedron transcription control sequences was produced in the following manner. A cDNA clone encoding the full-length alpha chain (a chain) of the ***human*** ***Fc*** .sub..epsilon. receptor was obtained from Dr. Jean-Pierre Kinet (Harvard University, Cambridge, Mass.). The cDNA clone included an about 1198 nucleotide insert,. . . denoted herein as SEQ ID NO:1. Translation of SEQ ID NO:1 indicates that nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.1198 encodes a full-length ***human*** ***Fc*** .sub..epsilon. receptor .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon.R.alpha..sub.257, having amino acid sequence SEQ ID. . . ID NO:1. The complement of SEQ ID NO:1 is represented herein by SEQ ID NO:3. The proposed mature protein (i.e., ***Fc*** .sub..epsilon.R.alpha. chain from which the signal sequence has been cleaved), denoted herein as PhFc.sub..epsilon.R.alpha..sub.232, contains about 232 amino acids which is. . .

DETD [0072] To produce a secreted form of the extracellular domain of the ***Fc*** .sub..epsilon.R a chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the ***Fc*** .sub..epsilon.R a chain encoded by nhFc.sub..epsilon.R.alpha..sub.1198 were removed as follows. A ***Fc*** .sub..epsilon.R a chain extracellular domain nucleic acid molecule-containing fragment of about 612 nucleotides was PCR amplified from nhFc.sub..epsilon.R.alpha..sub.1198 using a forward. . . EcoRI to produce nhFc.sub..epsilon.R.alpha..sub.612. Nucleic acid molecule

nhFc.sub..epsilon.R.alpha..sub.612 contained an about 591 nucleotide fragment encoding the extracellular domain of the ***human*** ***Fc*** .sub..epsilon.R a chain, extending from about nucleotide 107 to about nucleotide 697 of SEQ ID NO 1, denoted herein as nucleic. . . nucleic acid sequence denoted SEQ ID NO:10. Translation of SEQ ID NO:10 indicates that nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.612 encodes a ***Fc*** .sub..epsilon.R protein of about 197 amino acids, referred to herein as PhFc.sub..epsilon.R.alpha..sub.197, having amino acid sequence SEQ ID NO:11. Nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.612 encodes a secretable form of the ***human*** ***Fc*** .sub..epsilon.R a chain which does not possess a leader sequence, which is denoted herein as PhFc.sub..epsilon.R.alpha..sub.172 having amino acid sequence SEQ. . .

DETD [0077] This example describes the biotinylation of a recombinant ***human*** ***Fc*** .sub..epsilon.R alpha chain protein.

DETD [0081] The results shown in FIG. 1 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon.R detects the presence of canine IgE (closed circles) in a solid-phase assay in a similar manner as the control antibody. . .

DETD [0084] The results shown in FIG. 2 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon.R detects the presence of canine IgE antibodies that bind specifically to a common grass allergen or to a common tree. . .

DETD . . . in FIG. 3 indicate that canine IgE from a variety of dog sera are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R in a manner similar to using an antibody that binds specifically to canine IgE. The absence of detectable amounts of. . .

DETD . . . 4 indicate that canine IgE that binds specifically to a flea saliva antigen is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R.

DETD . . . IgE from dogs allergic to flea saliva and from dogs infected with heartworm are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the anti-IgE antibody and detected by ***Fc*** .sub..epsilon.R alpha chain is an epsilon isotype antibody and not another isotype.

DETD . . . indicate that canine IgE that binds specifically to flea saliva, contained in serum, is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of colorimetric signal in samples of heat inactivated serum indicates that antibody bound to the flea saliva protein and detected by ***Fc*** .sub..epsilon.R alpha chain is an epsilon isotype antibody.

DETD . . . feline IgE that binds specifically to crude homogenate of heartworm or Di33 protein is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R.

DETD . . . feline IgE from heartworm-infected cats that specifically binds to the heartworm antigen Di33 is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the Di33 protein and detected by ***Fc*** .sub..epsilon.R alpha chain is an epsilon isotype antibody.

DETD . . . be allergic to certain allergens specifically binds to certain plant and mite allergens is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R.

DETD [0106] This example describes detection of canine IgE in a solid-phase ELISA using basophilic cells transfected with ***human*** ***Fc*** .sub..epsilon.R alpha chain.

DETD [0107] Rat basophilic leukemia (RBL) cells transfected with a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon.R alpha chain (referred to herein as RBL-hFc.sub..epsilon.R cells; described in Miller et al., Science 244:334-337, 1989) were used to detect. . .

DETD [0110] The results shown in FIG. 10 indicate that canine IgE from heartworm-infected dogs (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon.R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon.R alpha chain on the RBL-h ***Fc*** .sub..epsilon.R cells is an epsilon isotype antibody. Similarly, the results shown in FIG. 11 indicate that canine IgE from dogs sensitized with flea saliva

(.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon.R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon.R alpha chain on the RBL-h ***Fc*** .sub..epsilon.R cells is an epsilon isotype antibody.

CLM What is claimed is:

106. An isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) alpha chain protein, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R alpha chain protein is conjugated to a ***detectable*** ***marker*** .

107. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 106, wherein said ***detectable*** ***marker*** is selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and. . .

108. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 106, wherein said ***detectable*** ***marker*** is selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, biotin-related compounds, avidin, avidin-related compounds and a. . .

109. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 106, wherein said ***Fc*** .sub..epsilon.R alpha chain protein is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule. . .

110. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 106, wherein said ***Fc*** .sub..epsilon.R alpha chain protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:6,. . .

111. An isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) alpha chain protein, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R alpha chain protein is conjugated to biotin.

112. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 1 11, wherein said ***Fc*** .sub..epsilon.R alpha chain protein is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule. . .

113. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 111, wherein said ***Fc*** .sub..epsilon.R alpha chain protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:6,. . .

114. An isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) alpha chain protein, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R alpha chain protein is conjugated to biotin, said protein comprising an amino acid sequence selected from the group consisting of. . .

115. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 114, wherein said ***Fc*** .sub..epsilon.R alpha chain protein is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule. . .

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TI Therapeutic polypeptides, nucleic acids encoding same, and methods of use

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PI US 2004067490 A1 20040408
AI US 2002-236392 A1 20020906 (10)
PRAI US 2002-390155P 20020619 (60)
US 2001-318765P 20010912 (60)
US 2002-357303P 20020215 (60)
US 2002-367753P 20020325 (60)
US 2002-369479P 20020402 (60)
US 2001-318120P 20010907 (60)
US 2001-318130P 20010907 (60)
US 2002-381672P 20020517 (60)
US 2001-318219P 20010907 (60)
US 2001-318430P 20010910 (60)
US 2001-322781P 20010917 (60)
US 2001-322816P 20010917 (60)
US 2001-323519P 20010919 (60)
US 2002-384012P 20020529 (60)
US 2001-323631P 20010920 (60)
US 2001-323636P 20010920 (60)
US 2002-360973P 20020228 (60)
US 2002-366131P 20020320 (60)
US 2001-324969P 20010925 (60)
US 2002-383651P 20020528 (60)
US 2001-325091P 20010925 (60)
US 2001-324990P 20010926 (60)
US 2002-381664P 20020517 (60)
US 2002-379532P 20020510 (60)
DT Utility
FS APPLICATION
LREP MINTZ, LEVIN, COHN,, FERRIS, GLOVSKY and POPEO, P.C., One Financial Center, Boston, MA, 02111
CLMN Number of Claims: 45
ECL Exemplary Claim: 1
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LN.CNT 36918
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed herein are nucleic acid sequences that encode novel polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies that immunospecifically bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the novel polypeptide, polynucleotide, or antibody specific to the polypeptide. Vectors, host cells, antibodies and recombinant methods for producing the polypeptides and polynucleotides, as well as methods for using same are also included. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel ***human*** nucleic acids and proteins.

AB . . . further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel ***human*** nucleic acids and proteins.

SUMM . . . invention includes the use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a ***human*** disease, the disease being selected from a pathology associated with a polypeptide with an amino acid sequence selected from the. . .

SUMM . . . prevention is desired in an amount sufficient to treat or prevent the pathology in the subject. The subject could be ***human*** .

SUMM . . . provides an antibody that binds immunospecifically to a NOVX polypeptide. The NOVX antibody may be monoclonal, humanized, or a fully ***human*** antibody. Preferably, the antibody has a dissociation constant for the binding of the NOVX polypeptide to the antibody less than. . .

SUMM . . . provides for the use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a ***human*** disease, associated with a NOVX polypeptide. Preferably the therapeutic is a NOVX antibody.

DRWD [0038] FIG. 1 is a Western blot showing expression of NOV30b (CG51117-05) immunoreactive polypeptide in ***human*** embryonic kidney 293 cells.

DETD . . . of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding ***human*** NOVX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in. . .

DETD [0072] The nucleotide sequences determined from the cloning of the ***human*** NOVX genes allows for the generation of probes and primers designed for use in identifying and/or cloning NOVX homologs in. . .

DETD [0073] Probes based on the ***human*** NOVX nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various. . .

DETD [0077] In addition to the ***human*** NOVX nucleotide sequences of SEQ ID NO:2n-1, wherein n is an integer between 1 and 127, it will be appreciated. . . that lead to changes in the amino acid sequences of the NOVX polypeptides may exist within a population (e.g., the ***human*** population). Such genetic polymorphism in the NOVX genes may exist among individuals within a population due to natural allelic variation.. . .

DETD . . . nucleic acid molecules encoding NOVX proteins from other species, and thus that have a nucleotide sequence that differs from a ***human*** SEQ ID NO:2n-1, wherein n is an integer between 1 and 127, are intended to be within the scope of. . . allelic variants and homologs of the NOVX cDNAs of the invention can be isolated based on their homology to the ***human*** NOVX nucleic acids disclosed herein using the ***human*** cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

DETD [0080] Homologs (i.e., nucleic acids encoding NOVX proteins derived from species other than ***human***) or other related sequences (e.g., paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular ***human*** sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

DETD . . . into a vector containing, e.g., a RNA pol III transcription unit from the smaller nuclear RNA (snRNA) U6 or the ***human*** RNase P RNA H 1. One example of a vector system is the GeneSuppressor RNA Interference kit (commercially available from. . .

DETD . . . derived from a mammal, more preferably from a rodent such as a rat or mouse, and most preferably from a ***human*** . Where used for therapeutic treatment, the cells are preferentially autologous, although non-autologous cell sources are also contemplated as within the. . .

DETD . . . Western blotting, ELISA, and the like. A subject sample of cells or tissues is taken from a mammal, preferably a ***human*** subject, suffering from a disease state. The NOVX ribopolynucleotide is used to produce siRNA constructs, that are specific for the. . .

DETD . . . chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of ***human*** antibody species.

DETD . . . of NOVX that is located on the surface of the protein, e.g., a hydrophilic region. A hydrophobicity analysis of the ***human*** NOVX protein sequence will indicate which regions of a NOVX polypeptide are particularly hydrophilic and, therefore, are likely to encode. . .

DETD . . . protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of ***human*** origin are desired, or spleen cells or lymph node cells are used if non- ***human*** mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such. . . Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and ***human*** origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture. . .

DETD . . . for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Manassas, Va. ***Human*** myeloma and mouse- ***human*** heteromyeloma cell lines also have been described for the production of ***human*** monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New. . .

DETD . . . antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for ***human*** heavy and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison, Nature 368,. . .

DETD [0184] The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or ***human*** antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the ***human*** against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab').sub.2 or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a ***human*** immunoglobulin, and contain minimal sequence derived from a non- ***human*** immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et. . . (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a ***human*** antibody. (See also U.S. Pat. No. 5,225,539.) In some instances, Fv framework residues of the ***human*** immunoglobulin are replaced by corresponding non- ***human*** residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR. . . and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non- ***human*** immunoglobulin and all or substantially all of the framework regions are those of a ***human*** immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (***Fc***), typically that of a ***human*** immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

DETD [0185] ***Human*** Antibodies

DETD [0186] Fully ***human*** antibodies essentially relate to antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from ***human*** genes. Such antibodies are termed " ***human*** antibodies", or "fully ***human*** antibodies" herein. ***Human*** monoclonal antibodies can be prepared by the trioma technique; the ***human*** B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce ***human*** monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). ***Human*** monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using ***human*** hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming ***human*** B-cells with Epstein Barr Virus in vitro (see Cole, el al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R.. . .

DETD [0187] In addition, ***human*** antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, ***human*** antibodies can be made by introducing ***human*** immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin

genes have been partially or completely inactivated. Upon challenge, ***human*** antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody. . .

DETD [0188] ***Human*** antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully ***human*** antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding ***human*** heavy and light chain immunoglobulins are inserted into the host's genome. The ***human*** genes are incorporated, for example, using yeast artificial chromosomes containing the requisite ***human*** DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals. . . the Xenomouse.TM. as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully ***human*** immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example,. . . immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with ***human*** variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs. . .

DETD [0190] A method for producing an antibody of interest, such as a ***human*** antibody, is disclosed in U.S. Pat. No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding. . .

DETD [0195] Bispecific antibodies are monoclonal, preferably ***human*** or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the. . .

DETD . . . the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal ***human*** T cells, as well as trigger the lytic activity of ***human*** cytotoxic lymphocytes against ***human*** breast tumor targets.

DETD . . . to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or ***Fc*** receptors for IgG (***Fc*** .gamma.R), such as ***Fc*** .gamma.RI (CD64), ***Fc*** .gamma.RII (CD32) and ***Fc*** .gamma.RIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be. . .

DETD [0221] Antibodies of the invention, including polyclonal, monoclonal, humanized and fully ***human*** antibodies, may used as therapeutic agents. Such agents will generally be employed to treat or prevent a disease or pathology. . .

DETD . . . immunoassays are described, for example in "ELISA: Theory and Practice: Methods in Molecular Biology", Vol. 42, J. R. Crowther (Ed.) ***Human*** Press, Totowa, N.J., 1995; "Immunoassay", E. Diamandis and T. Christopoulus, Academic Press, Inc., San Diego, Calif., 1996; and "Practice and. . .

DETD [0251] The host cells of the invention can also be used to produce non- ***human*** transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which NOVX protein-coding sequences have been introduced. Such host cells can then be used to create non- ***human*** transgenic animals in which exogenous NOVX sequences have been introduced into their genome or homologous recombinant animals in which endogenous. . . NOVX protein and for identifying and/or evaluating modulators of NOVX protein activity. As used herein, a "transgenic animal" is a non- ***human*** animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non- ***human*** primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of. . . one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non- ***human*** animal, preferably a mammal, more preferably a mouse, in which an endogenous NOVX gene has been altered by homologous recombination. . .

DETD . . . fertilized oocyte (e.g., by microinjection, retroviral

infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The ***human*** NOVX cDNA sequences, i.e., any one of SEQ ID NO:2n-1, wherein n is an integer between 1 and 127, can be introduced as a transgene into the genome of a non- ***human*** animal. Alternatively, a non- ***human*** homolog of the ***human*** NOVX gene, such as a mouse NOVX gene, can be isolated based on hybridization to the ***human*** NOVX cDNA (described further supra) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in. . .

DETD . . . or substitution has been introduced to thereby alter, e.g., functionally disrupt, the NOVX gene. The NOVX gene can be a ***human*** gene (e.g., the cDNA of any one of SEQ ID NO:2n-1, wherein n is an integer between 1 and 127), but more preferably, is a non- ***human*** homolog of a ***human*** NOVX gene. For example, a mouse homolog of ***human*** NOVX gene of SEQ ID NO:2n-1, wherein n is an integer between 1 and 127, can be used to construct. . .

DETD [0257] Clones of the non- ***human*** transgenic animals described herein can also be produced according to the methods described in Wilmut, et al., 1997. Nature 385:. . .

DETD . . . examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% ***human*** serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and. . .

DETD . . . detecting the induction of a reporter gene (comprising a NOVX-responsive regulatory element operatively linked to a nucleic acid encoding a ***detectable*** ***marker*** , e.g., luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

DETD . . . thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual ***human*** chromosomes. Only those hybrids containing the ***human*** gene corresponding to the NOVX sequences will yield an amplified fragment.

DETD [0298] Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., ***human*** and mouse cells). As hybrids of ***human*** and mouse cells grow and divide, they gradually lose ***human*** chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which ***human*** cells can, the one ***human*** chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single ***human*** chromosome or a small number of ***human*** chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific ***human*** chromosomes. See, e.g., D'Eustachio, et al., 1983. Science 220: 919-924. Somatic cell hybrids containing only fragments of ***human*** chromosomes can also be produced by using ***human*** chromosomes with translocations and deletions.

DETD . . . to get good results at a reasonable amount of time. For a review of this technique, see, Verma, et al., ***HUMAN*** CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

DETD . . . obtain such identification sequences from individuals and from tissue. The NOVX sequences of the invention uniquely represent portions of the ***human*** genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in. . .

DETD . . . e.g., DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. See, e.g., Cronin, et al., 1996, ***Human*** Mutation 7: 244-255; Kozal, et al., 1996, Nat. Med. 2: 753-759. For example, genetic mutations in NOVX can be identified. . .

DETD . . . model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in ***human*** subjects. Similarly, for in vivo testing, any of the animal model system known in the art may be used prior to administration to ***human*** subjects.

DETD . . . 1D

NOV1a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	Residues	Matched Region	Similarities for the Matched Region	Expect Value
AAU74674	***Human*** fibronectin protein - Homo sapiens, 2324 aa. [WO200177327-A1, 18-OCT-2001]	1 . . . 2320 5 . . . 2324. 9 . . . 2328	2320/2320 (100%) 2320/2320 (100%) 2320/2320 (100%)		0.0 0.0 0.0
AAR92778	***Human*** fibronectin - Homo sapiens, 2324 aa. [WO9604304-A1, 15-FEB-1996]	1 . . . 2320 5 . . . 2324	2318/2320 (99%) 2318/2320 (99%)		0.0 0.0
AAP70373	***Human*** fibronectin gene product - Homo sapiens, 2327 aa. [EP207751-A, 07-JAN-1987]	1 . . . 2320 8 . . . 2327	2318/2320 (99%) 2318/2320 (99%)		0.0 0.0
AAM38649	***Human*** polypeptide SEQ ID NO 1794 - Homo sapiens,	1 . . . 2320 36	2316/2320 (99%)		0.0
DETD	 2320	2318/2351 (98%)			0.0
	(Cold-insoluble globulin) (CIG) - Homo sapiens (***Human***), 2386 aa.	36 . . . 2386	2318/2351 (98%)		
FNHU	fibronectin precursor [validated] - ***human*** , 2386 aa.	1 . . . 2320 36 . . . 2386	2318/2351 (98%) 2318/2351 (98%)		0.0
E981236	FN PLASMID PFHDEL1	1 . . . 1946	1703/2026 (84%)		0.0
. . .					
DETD	. . . 311	311/311 (100%)			0.0
	(Hepatic lectin H2) (ASGP-R) (ASGPR) - Homo sapiens (***Human***), 311 aa.	1 . . . 311	311/311 (100%)		
P24721	Asialoglycoprotein receptor 2 (Hepatic lectin 2) (MHL-2) (Rat), 301 aa.	1 . . . 307 1	198/307 (64%) - Rattus norvegicus		e-114
AAH32130	Asialoglycoprotein receptor 1 - Homo sapiens (***Human***), 291 aa.	1 . . . 301 1 . . . 278	173/301 (57%) 213/301 (70%)		e-103
DETD	. . . 3D				

Geneseq Results for NOV3a

NOV3a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	Residues	Matched Region	Similarities for the Matched Region	Expect Value
AAE02462	***Human*** Mcl-1 protein - Homo sapiens, 350 aa. [WO200136594-A1, 25-MAY-2001]	1 . . . 350 1 . . . 350	350/350 350/350 (100%)		0.0
AAR68814	***Human*** mcl-1 gene product - Homo sapiens, 350 aa.	1 . . . 350 1	349/350 protein sequence		0.0
SEQ	1 . . . 331	289/350 (82%)			

ID NO: 570 - Mus musculus, 331 aa. [WO200188188-A2, 22-NOV-2001]

AAE02463 ***Human*** Mcl-1s/deltaTM variant protein - Homo 1 . . . 230 230/230 (100%) e-129 1 . . . 230 230/230 (100%)

. . .

DETD . . . for NOV3a

Protein Accession Number	Protein/Organism/Length	NOV3a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
A47476	BCL2 homolog MCL1 - ***human*** , 350 aa.	1 . . . 350	350/350 (100%)	0.0
Q9UNJ1	Myeloid cell differentiation. . . cell leukemia protein 1) (Myeloid cell leukemia sequence 1) (BCL2-related) - Homo sapiens (***Human***), 350 aa.	1 . . . 350	350/350 (100%)	1 . . . 350
Q07820	Induced myeloid leukemia cell differentiation protein Mcl-1 - Homo sapiens (***Human***), 350 aa.	1 . . . 350	349/350 (99%)	0.0
Q9Z1P3	Mcl-1 protein - Rattus norvegicus (Rat), 330 aa.	1 . . . 350	348/350 (99%)	e-144
		1 . . . 350	349/350 (99%)	
		1 . . . 350	271/350 (77%)	
		1 . . .		

DETD . . . Results for NOV4a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV4a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABB89547	***Human*** polypeptide SEQ ID NO 1923 - Homo sapiens, 244 aa. [WO200190304-A2, 29-NOV-2001]	1 . . . 200	199/200 (99%)	e-113
AAM40701	***Human*** polypeptide SEQ ID NO 5632 - Homo sapiens, 316 aa. [WO200153312-A1, 26-JUL-2001]	73 . . . 272	200/200 (99%)	e-113
AAM38915	***Human*** polypeptide SEQ ID NO 2060 - Homo sapiens, 341 aa. [WO200153312-A1, 26-JUL-2001]	98 . . . 297	200/200 (99%)	e-113
ABB11939	***Human*** secreted protein homolog, SEQ ID NO: 2309 - Homo sapiens, 274 aa. [WO200157188-A2, 09-AUG-2001]	31 . . . 230	200/200 (99%)	e-113
ABG02475	Novel ***human*** diagnostic protein #2466 - Homo	20 . . . 108	82/89 (92%)	2e-42
		209 . . . 297	85/89 (95%)	

. . .

DETD . . . 1 . . . 200 199/200 (99%) e-112

	2310034L04 gene - Homo sapiens (***Human***), 244 aa.	1 . . . 200	200/200 (99%)	
Q99KZ9	Hypothetical 32.8 kDa protein - Mus musculus (Mouse), 289	26 . . . 200	169/177 (95%)	2e-92
		69. . .		

DETD . . . Results for NOV5a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV5a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB36613	***Human*** FLEXHT-35 protein sequence SEQ ID NO: 35 - Homo sapiens, 330 aa. [WO200070047-A2, 23-NOV-2000]	1 . . . 330	302/330 (91%)	e-174
		1 . . . 330	312/330 (94%)	
ABG13115	Novel ***human*** diagnostic protein #13106 - Homo sapiens, 425 aa. [WO200175067-A2, 11-OCT-2001]	1 . . . 297	274/297 (92%)	e-158
		23 . . . 319	284/297 (95%)	
ABG09575	Novel ***human*** diagnostic protein #9566 - Homo sapiens, 379 aa. [WO200175067-A2, 11-OCT-2001]	1 . . . 330	259/379 (68%)	e-134
		1 . . . 379	277/379 (72%)	
ABG13114	Novel ***human*** diagnostic protein #13105 - Homo sapiens, 490 aa. [WO200175067-A2, 11-OCT-2001]	1 . . . 297	227/346 (65%)	e-113
		1 . . . 346	245/346 (70%)	
AAU33207	Novel ***human*** secreted protein #3698 - Homo sapiens, 352	33 . . . 297	209/266 (78%)	e-108
		8 . . . 246	. .	
DETD	. . . 1 . . . 330 302/330 (91%) e-174			
	5730409G15 gene - Homo sapiens (***Human***), 330 aa.	1 . . . 330	312/330 (94%)	
Q96S85	Hypothetical 33.0 kDa protein - Homo sapiens (***Human***), 296 aa.	1 . . . 330	272/330 (82%)	e-152
		1 . . . 296	282/330 (85%)	
Q9CS89	5730409G15Rik protein -	1 . . . 298	214/298 (71%)	e-117
. . . clone		1 . . . 159	113/159 (71%)	1e-53
	TRACH2010771 - Homo sapiens (***Human***), 153 aa.	1 . . . 125	116/159 (72%)	
Q9NVL1	CDNA FLJ10661 fis, clone	1 . . . 114	79/114 (69%)	4e-33
	NT2RP2006106 - Homo sapiens (***Human***), 165 aa.	1 . . . 87	83/114 (72%)	

DETD . . . Results for NOV6a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV6a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB36613	***Human*** FLEXHT-35 protein sequence SEQ ID NO: 35 - Homo sapiens, 330 aa. [WO200070047-A2, 23-NOV-2000]	1 . . . 296	271/330 (82%)	e-151
		1 . . . 330	281/330 (85%)	
ABG13115	Novel ***human*** diagnostic protein #13106 - Homo sapiens, 425 aa. [WO200175067-A2, 11-OCT-2001]	1 . . . 263	243/297 (81%)	e-135
		23 . . . 319	253/297 (84%)	

ABG09575 Novel ***human*** diagnostic 19 . . . 296 220/299 (73%) e-114
protein #9566 - Homo 89 . . . 379 233/299 (77%)
sapiens, 379 aa.
[WO200175067-A2, 11-
OCT-2001]
ABG13114 Novel ***human*** diagnostic 19 . . . 263 188/266 (70%) 7e-94
protein #13105 - Homo 89 . . . 346 203/266 (75%)
sapiens, 490 aa.
[WO200175067-A2, 11-
OCT-2001]
AAU33207 Novel ***human*** secreted protein 33 . . . 263 183/242 (75%) 9e-92
#3698 - Homo sapiens, 352 8 . . . 246. . .

DETD . . . Number	Protein/Organism/Length	Residues	Portion	Expect Value
Q96S85	Hypothetical 33.0 kDa protein -	1 . . . 296	272/296 (91%)	e-157
	Homo sapiens (***Human***), 296 aa.	1 . . . 296	282/296 (94%)	
Q96G04	Similar to RIKEN cDNA	1 . . . 296	271/330 (82%)	e-151
	5730409G15 gene - Homo	1 . . . 330	281/330 (85%)	
	sapiens (***Human***), 330 aa.			
Q9CS89	5730409G15Rik protein -	1 . . . 264	189/298 (63%)	5e-98
	Mus musculus (Mouse), 319	1	clone	
1 . . . 125	113/125 (90%)	6e-59		
	TRACH2010771 - Homo	1 . . . 125	116/125 (92%)	
	sapiens (***Human***), 153 aa.			
AAH32519	Similar to hypothetical	1 . . . 70	51/70 (72%)	7e-20
	protein FLJ10661 - Homo sapiens	1 . . . 66	58/70 (82%)	
	(***Human***), 131 aa.			

DETD . . . ORF Stop: TAA at 1438

SEQ ID NO: 26 414 aa MW at 45936.7kD

NOV7a MKKHSARVAPLSACNSPVLTLTKVEGEERPRDSPGPAEAQAPAGVEAGGRASRR CWTCSRA

CG137717-01
Protein Sequence QLKKIFWGVAVVLCVCSSWAGSTQLAKLTFRKFDAPFTLTWFATNWNFLFFPLY YVGHVCK

STEKQSVKQRYRECCRFFGDNGLTLKVFFTKAAPFGVLWTLTNYLYLHAIKKIN TTDVSVL

FCCNKAFVFLLSWIVLRDRFMGVIVAAILAIAGIVMMTYADGFHSHSVI *** GIALVVASASVL***

FKLLLCSAKFGEAALFLSILGVFNILFITCIPIILYFTKVEYWSSFDDIPWCNL CGFSVLL

LAFNIVLNFGIAVTYPTLMSLGIVLSIPVNAVIDHYTSQIVFNOVRVIAIIIIG LGFLLLL

LPEEWDVWLIKLLTRLKVRKKEEPAEGAADLSSGPQSKNRRARPSFAR

DETD . . . Results for NOV7a

Residues/ Geneseq Identifier	Identities/ Protein/Organism/Length [Patent #, Date]	NOV7a Match Residues	Similarities for the Matched Region	Expect Value
ABG16671	Novel ***human*** diagnostic	5 . . . 284	160/329 (48%)	2e-80
	protein #16662 - Homo sapiens, 531 aa.	168 . . . 492	208/329 (62%)	

[WO200175067-A2, 11-
OCT-2001]
ABB89266 ***Human*** polypeptide SEQ ID 1 . . . 134 134/134
(100%) 1e-76
NO 1642 - Homo sapiens, 1 . . .. . . for measuring
1 . . . 77 77/77 (100%)
placental gene expression -
Homo sapiens, 77 aa.
[WO200157272-A2, 09-
AUG-2001]
AAM76340 ***Human*** bone marrow 338 . . . 414 77/77
(100%) 5e-37
expressed probe encoded 1 . . . 77 77/77 (100%)
protein SEQ ID NO: 36646 -
Homo sapiens, 77 aa.
[WO200157276-A2, 09-
AUG-2001]
AAM63526 ***Human*** brain expressed 338 . . . 414 77/77
(100%) 5e-37
single exon probe encoded 1 . . . 77 77/77. . .
DETD . . . clone 27 . . . 414 387/395 (97%) 0.0
BRHIP2018369 - Homo sapiens 96 . . . 490 387/395 (97%)
(***Human***), 490 aa.
Q9JJG8 Brain cDNA, clone MNCb- 114 . . . 406 179/300 (59%)
1e-99
0335 - Mus musculus 26 .. . .
DETD . . . [Patent #, Date] Residues Matched Region
Value

AAB31584 Amino acid sequence of a 1 . . . 257 257/257 (100%)
e-155
human ***Fc*** ***epsilon*** ***receptor***
1 . . . 257 257/257 (100%)
alpha-chain - Homo sapiens,
257 aa. [WO200104310-A1,
18-JAN-2001]
AAB74667 ***Human*** immunoglobulin E 1 . . . 257 257/257
(100%) e-155
receptor I alpha subunit 1 . . . 257 257/257 (100%)
protein - Homo sapiens, 257
aa. [WO200111010-A2, 15-
FEB-2001]
AAY96230 ***Human*** ***Fc*** receptor, 1 . . . 257
257/257 (100%) e-155
FcepsilonRIa - Homo 4 . . . 260 257/257
(100%)
sapiens, 260 aa.
[EP1006183-A1, 07-JUN-
2000]
AAW61190 The alpha chain of a ***Fc*** 1 . . . 257 257/257
(100%) e-155
epsilon receptor - Homo 1 . . . 257 257/257 (100%)
sapiens, 257 aa.
[WO9823964-A1, 04-JUN-
1998]
AAW24066 Alpha subunit of ***human*** high 1 . . . 257 257/257
(100%) e-155
affinity receptor for IgE 1 . . . 257 257/257 (100%)
(***human*** ***FcERI***) - Homo
sapiens, 257 aa.
[US5639660-A, 17-JUN-
1997]
DETD . . . 1 . . . 257 257/257 (100%) e-154
immunoglobulin epsilon 1 . . . 257 257/257 (100%)
receptor alpha-subunit
precursor (***FcERI***) (IgE ***Fc***
receptor, alpha-subunit) (***Fc*** -
epsilon RI-alpha) - Homo
sapiens (***Human***), 257 aa.
AAH15195 ***Fc*** IgE, high affinity I, 1 . . . 257 256/257
(99%) e-154

```
                receptor for, alpha              1 . . . 257  256/257 (99%)
                polypeptide - Homo sapiens
                (  ***Human***  ), 257 aa.
CAC28464        Sequence 4 from Patent          26 . . . 257  232/232 (100%)
        e-139
                WO0104310 - Homo sapiens         1 . . . 232  232/232 (100%)
                (  ***Human***  ), 232 aa (fragment).
CAC28471        Sequence 26 from Patent          1 . . . 197  197/197 (100%)
        e-117
                WO0104310 - Cloning vector       1.  .  .
DETD    .  .  .  Results for NOV9a
                                                 NOV9a              Identities/
                                                 Residues/          Similarities for
Geneseq         Protein/Organism/Length          Match              the Matched
        Expect
Identifier      [Patent #, Date]                 Residues           Region
        Value

AAR82244          ***Human***   fibrinogen A-alpha          1 . . . 644
        643/644 (99%)   0.0
                chain protein - Homo sapiens,     1 . . . 644.  .  .  . 644
        641/644 (99%)   0.0
                643 aa. [WO9416085-A, 21-         1 . . . 643     641/644 (99%)
                JUL-1994]
AAY82891        AlphaE subunit of   ***human***             20 . . . 641
        615/626 (98%)   0.0
                fibrinogen - Homo sapiens,        1 . . . 626     616/626 (98%)
                847.  .  .  .  . 644    416/435 (95%)   0.0
                Homo sapiens, 1336 aa.           910 . . . 1336   417/435 (95%)
                [WO9416085-A, 21-JUL-
                1994]
AAB54135          ***Human***   pancreatic cancer           1 . . . 307
        301/307 (98%)   e-176
                antigen protein sequence SEQ     22 . . . 328    301/307.  .  .
DETD    .  .  .  .  .  .  644  644/644 (100%)       0.0
                precursor, short splice form     1 . . . 644  644/644 (100%)
                [validated] -   ***human***  , 644 aa.
P02671          Fibrinogen alpha/alpha-E          1 . . . 641  634/645 (98%)
        0.0
                chain precursor [Contains:        1 . . . 645  635/645 (98%)
                Fibrinopeptide A] - Homo
                sapiens (  ***Human***  ), 866 aa.
P02672          Fibrinogen alpha chain           20 . . . 644  375/633 (59%)
        0.0
                [Contains: Fibrinopeptide A] -    4 . . .  .  .
DETD    .  .  .  . 235  187/187 (100%)       e-107
                0-C2-FL - Homo sapiens,          1 . . . 187  187/187 (100%)
                187 aa. [WO9940189-A2,
                12-AUG-1999]
AAE01707          ***Human***   gene 5 encoded          70 . . . 235  166/166
        (100%)       1e-92
                secreted protein HHBCS39,        1 . . . 166  166/166 (100%)
                SEQ ID NO: 119 - Homo
                sapiens, 166 aa.
                [WO200134767-A2,
                17-MAY-2001]
AAE01676          ***Human***   gene 5 encoded          70 . . . 235  166/166
        (100%)       1e-92
                secreted protein HHBCS39,        1 . . . 166  166/166 (100%)
                SEQ ID NO: 88 - Homo
                sapiens, 166 aa.
                [WO200134767-A2,
                17-MAY-2001]
AAY65073          ***Human***   5' EST related           1 . . . 59    56/59
        (94%)        5e-24
                polypeptide SEQ ID               1 . . . 59    56/59 (94%)
                NO: 1234 - Homo sapiens, 59
                aa. [WO9953051-A2,
                21-OCT-1999]
AAG01373          ***Human***   secreted protein,       49 . . . 184   49/137
        (35%)        7e-11
```

SEQ ID NO: 5454 - Homo 1 . . . . . .
DETD . . . for the Expect

Number	Protein/Organism/Length	Residues	Matched Portion	Value
AAM88866	MTLC - Homo sapiens	1 . . . 235	235/235 (100%)	e-134
	(***Human***), 235 aa.	1 . . . 235	235/235 (100%)	
Q9H763	CDNA: FLJ21269 fis, clone	1 . . . 235	234/235 (99%)	e-133
	COL01745 - Homo sapiens (***Human***), 235 aa.	1 . . . 235	235/235 (99%)	
CAD39158	Hypothetical protein - Homo	32 . . . 235	204/204 (100%)	e-115
	sapiens (***Human***), 204 aa	1 . . . 204	204/204 (100%)	
	(fragment).			
Q8TBE8	Similar to RIKEN cDNA	49 . . . 235	186/187 (99%)	e-105
	1110020B04 gene - Homo sapiens (***Human***), 187 aa.	1 . . . 187	186/187 (99%)	
Q8R411	MT-MC1 - Mus musculus	49 . . . 235	160/188 (85%)	4e-90
	(Mouse), 188 aa.	1		

DETD . . . Results for NOV11a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV11a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAM79290	***Human*** protein SEQ ID NO	42 . . . 299	256/258	
(99%)	e-154			
	1952 - Homo sapiens, 258 aa. [WO200157190-A2, 09-AUG-2001]	1 . . . 258	257/258 (99%)	
ABB89913	***Human*** polypeptide SEQ ID	1 . . . 299	238/299	
(79%)	e-149			
	NO 2289 - Homo sapiens, 375 aa. [WO200190304-A2, 29-NOV-2001]	77 . . . 375	269/299 (89%)	
AAB74699	***Human*** membrane associated	1 . . . 299	238/299	
(79%)	e-149			
	protein MEMAP-5 - Homo sapiens, 375 aa. [WO200112662-A2, 22-FEB-2001]	77 . . . 375	269/299 (89%)	
AAM79634	***Human*** protein SEQ ID NO	1 . . . 299	238/299	
(79%)	e-149			
	3280 - Homo sapiens, 379 aa. [WO200157190-A2, 09-AUG-2001]	81 . . . 379	269/299 (89%)	
AAM78650	***Human*** protein SEQ ID NO	1 . . . 299	238/299	
(79%)	e-149			
	1312 - Homo sapiens, 375 aa.	77		

DETD . . . clone 42 . . . 299 256/258 (99%) e-153

	COL04219 - Homo sapiens (***Human***), 258 aa.	1 . . . 258	257/258 (99%)	
Q91VH1	Hypothetical 42.4 kDa protein - Mus musculus (Mouse), 375	1 . . . 299	238/299 (79%)	e-149
		77. 299	238/299 (79%)	e-149
	(Hypothetical 42.6 kDa protein) - Homo sapiens (***Human***), 375 aa.	77 . . . 375	269/299 (89%)	
Q9Y360	CGI-45 protein - Homo sapiens (***Human***), 370 aa.	1 . . . 292	236/292 (80%)	e-147
		77 . . . 368	264/292 (89%)	
Q9CZA0	2810031L11 Rik protein - Mus	1 . . . 276	211/276. . .	

DETD . . .

TABLE 12C

Geneseq Results for NOV12a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	Residues/ Match Residues	Similarities for the Matched Region	Expect Value
AAM49113	***Human*** dendritic cell membrane protein Siglec-9 - Unidentified, 463 aa. [WO200078961-A1, 28-DEC-2000]	1 . . . 165	164/165 (99%)	5e-97
AAB87568	***Human*** PRO1302 - Homo sapiens, 463 aa. [WO200078961-A1, 28-DEC-2000]	1 . . . 165	164/165 (99%)	5e-97
DETD	. . . 1 . . . 165	164/165 (99%)		1e-96
	immunoglobulin-like lectin-9 - Homo sapiens (***Human***), 463 aa.	1 . . . 165	164/165 (99%)	
Q9Y336	OB-binding protein-like protein (Sialic acid-binding lectin) - Homo sapiens (***Human***), 463 aa.	1 . . . 165 1 . . . 165	164/165 (99%) 164/165 (99%)	1e-96
Q9BYI9	FOAP-9 - Homo sapiens (***Human***), 463 aa.	1 . . . 165 1 . . . 165	163/165 (98%) 164/165 (98%)	4e-96
Q9Y286	QA79 membrane protein, allelic variant AIRM-1B precursor - Homo sapiens (***Human***), 467 aa.	1 . . . 165 2 . . . 169	132/169 (78%) 138/169 (81%)	3e-68
Q9Y502	QA79 membrane protein, splice product AIRM-2 precursor - Homo sapiens (***Human***), 374 aa.	1 . . . 140 2 . . . 144	109/144 (75%) 115/144 (79%)	6e-55

DETD . . . Results for NOV13a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV13a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAE06730	***Human*** CASB765 protein - Homo sapiens, 311 aa. [WO200157077-A1, 09-AUG-2001]	25 . . . 264 1 . . . 311	208/318 215/318 (67%)	e-100
AAU81960	***Human*** PRO536 - Homo sapiens, 313 aa. [WO200109327-A2, 08-FEB-2001]	25 . . . 263 1 . . . 301	174/302 187/302 (61%)	8e-79
AAB65173	***Human*** PRO536 (UNQ337) protein sequence SEQ ID NO: 97 - Homo sapiens, 313 aa. [WO200073454-A1, 07-DEC-2000]	25 . . . 263 1 . . . 301	174/302 187/302 (61%)	8e-79
AAB94830	***Human*** protein sequence SEQ ID NO: 15991 - Homo sapiens, 313 aa. [EP1074617-A2, 07-FEB-2001]	25 . . . 263 1 . . . 301	174/302 187/302 (61%)	8e-79
AAU12370	***Human*** PRO536 polypeptide sequence - Homo sapiens,	25 . . . 263 1 . . . 301	174/302 187/302. . .	8e-79
DETD	. . . 25 . . . 263	174/302 (57%)		2e-78
	ZSIG11 precursor - Homo sapiens (***Human***), 313 aa.	1 . . . 301	187/302 (61%)	

```
CAC25002        Sequence 46 from Patent          25 . . . 263 173/302 (57%)
       2e-76
                WO0100806 precursor -             1 . . . 300  186/302 (61%)
                Homo sapiens (  ***Human***  ), 312
                aa.
Q9UKD7          Hypothetical 9.7 kDa protein -  183 . . . 263  67/81 (82%)
       4e-30
                Homo sapiens (  ***Human***  ), 93         1 . . . 81    69/81
       (84%)
                aa.
DETD    .  .  .  for NOV14a
                                                NOV14a        Identities/
                                                Residues/     Similarities for
Geneseq         Protein/Organism/Length         Match         the Matched
Identifier      [Patent #, Date]                Residues      Region
       Expect Value

AAE09454          ***Human***   sbg72825FOLATEa          1 . . . 243  243/250
       (97%)   e-156
                protein - Homo sapiens, 250    1 . . . 250  243/250 (97%)
                aa. [WO200160850-A1,
                23-AUG-2001]
AAB50286          ***Human***   folate receptor II       4 . . . 222  130/222
       (58%)   8e-82
                protein SEQ ID NO: 6 -         5 . . . 226  158/222 (70%)
                Homo sapiens, 255 aa.
                [WO200071754-A1,
                30-NOV-2000]
ABG19167        Novel   ***human***   diagnostic        19 . . . 222  120/207
       (57%)   7e-70
                protein #19158 - Homo          29 . . . 235  144/207 (68%)
                sapiens, 248 aa.
                [WO200175067-A2,
                11-OCT-2001]
ABG04155        Novel   ***human***   diagnostic        46 . . . 242  101/205
       (49%)   5e-54
                protein #4146 - Homo           1 . . . 204  128/205 (62%)
                sapiens, 206 aa.
                [WO200175067-A2,
                11-OCT-2001]
ABG19166        Novel   ***human***   diagnostic        19 . . . 153   66/151
       (43%)   9e-30
                protein #19157 - Homo          27 . . . 176   81/151 (52%)
. . .
DETD    .  .  .  (67%)
            receptor 1) (Folate receptor,
            adult) (Adult folate-binding
            protein) (FBP) (Ovarian
            tumor-associated antigen
            MOv18) (KB cells FBP) -
            Homo sapiens (  ***Human***  ), 257
            aa.
Q9XSH1      Membrane-bound folate           7 . . . 239  138/240 (57%)   4e-84
            binding protein - Sus scrofa    8 . . . . . 222  129/204 (63%)
       5e-82
            precursor (FR-gamma) (Folate   27 . . . 230  152/204 (74%)
            receptor 3) - Homo sapiens
            (  ***Human***  ), 243 aa.
P35846      Folate receptor alpha           7 . . . 242  135/242 (55%)   7e-82
            precursor (FR-alpha) (Folate   10 . . . . . .
DETD    .  .  .  15C


Geneseq Results for NOV15a
                                                Residues/     Identities/
Geneseq         Protein/Organism/Length         Match         Similarities for the
       Expect
Identifier      [Patent #, Date]                Residues      Matched Region
       Value

AAU96185          ***Human***   secreted protein,        1 . . . 542  542/542
```

(100%) 0.0
SEQ ID No 87 - Homo 6 . . . 547 542/542 (100%)
sapiens, 547 aa.
[WO200224721-A1,
28-MAR-2002]
ABG27904 Novel ***human*** diagnostic 26 . . . 542 515/517
(99%) 0.0
protein #27895 - Homo 74 . . . 590 515/517 (99%)
sapiens, 590 aa.
[WO200175067-A2,
11-OCT-2001]
AAU83597 ***Human*** PRO protein, Seq ID 4 . . . 542 372/540
(68%) 0.0
No 12 - Homo sapiens, 544 9 . . . 544 441/540 (80%)
aa. [WO200208288-A2,
31-JAN-2002]
AAU96219 ***Human*** secreted protein, 1 . . . 298 291/298
(97%) e-170
SEQ ID No 121 - Homo 6 . . . 303 291/298 (97%)
sapiens, 303 aa.
[WO200224721-A1,
28-MAR-2002]
AAB74709 ***Human*** membrane associated 4 . . . 273 220/270
(81%) e-129
protein MEMAP-15 - Homo 9 . . . 277 245/270. . .
DETD . . . kDa 138 . . . 542 404/405 (99%) 0.0
protein - Homo sapiens 1 . . . 405 404/405 (99%)
(***Human***), 405 aa.
Q9CX72 4432416J03Rik protein - Mus 6 . . . 542 339/539 (62%)
0.0
musculus (Mouse), 558 aa. 24 .. . . clone 2 . .
. 292 205/291 (70%) e-116
STM00905 - Homo sapiens 18 . . . 308 239/291 (81%)
(***Human***), 365 aa.
Q969Y0 CDNA FLJ30102 fis, clone 18 . . . 542 168/543 (30%)
3e-69
BNGH41000137, weakly 19 . . . 555 287/543 (51%)
similar to brush border 61.9 kDa
protein precursor
(Unknown) (Protein for
MGC: 15606) - Homo sapiens
(***Human***), 559 aa.
DETD . . . for NOV16a

Geneseq Identifier Expect Value	Protein/Organism/Length [Patent #, Date]	NOV16a Residues/ Match Residues	Identities/ Similarities for the Matched Region
AAM00776 (97%) e-118	***Human*** bone marrow protein, SEQ ID NO: 139 - Homo sapiens, 211 aa. [WO200153453-A2, 26-JUL-2001]	181 . . . 391 1 . . . 211	205/211 206/211 (97%)
AAM00889 (96%) e-113	***Human*** bone marrow protein, SEQ ID NO: 365 - Homo fragment SEQ ID NO: 38498 - Arabidopsis thaliana, 476 aa. [EP1033405-A2, 06-SEP-2000]	170 . . . 368 3 . .. . . e-110 31 . . . 462	193/199 296/470 (62%)
AAB42327 (94%) e-100	***Human*** ORFX ORF2091 polypeptide sequence SEQ	295 . . . 489 2 . . . 192	185/195 187/195 (95%)

. . .
DETD . . . similar 1 . . . 497 495/501 (98%)
to Mus musculus cAMP
inducible 2 protein (Ci2)
mRNA - Homo sapiens
(***Human***), 501 aa.

```
Q9WU81          cAMP inducible 2 protein -      1 . . . 501  435/501 (86%)
       0.0
                Mus musculus (Mouse), 501       1.  .  .  .  .  . 497  461/501 (91%)
                aa.
Q8TEM2          FLJ00171 protein - Homo         1 . . . 346  346/346 (100%)
       0.0
                sapiens (  ***Human***  ), 396 aa       12 . . . 357  346/346
       (100%)
                (fragment).
Q8R070          Similar to solute carrier       5 . . . 489  308/516.  .  .
DETD   .  .  .  Results for NOV17a
                                                NOV17a
                                                Residues/     Identities/
Geneseq         Protein/Organism/Length         Match         Similarities for the
       Expect
Identifier      [Patent #, Date]                Residues      Matched Region
       Value

ABB07822          ***Human***   notch agonist ligand -  1 . . . 1218  1218/1218
       (100%)       0.0
                Homo sapiens, 1218 aa.          1 . . . 1218  1218/1218 (100%)
                [WO200218544-A2,
                07-MAR-2002]
AAW87894          ***Human***   JAGGED1 protein -       1 . . . 1218  1218/1218
       (100%)       0.0
                Homo sapiens, 1218 aa.          1 . . . 1218  1218/1218 (100%)
                [WO9858958-A2,
                30-DEC-1998]
AAW44301          ***Human***   serrate 1 - Homo        1 . . . 1218  1218/1218
       (100%)       0.0
                sapiens, 1218 aa.               1 . . . 1218.  .  .  0.0
                expressed in breast cancer      1 . . . 1218  1217/1218 (99%)
                tissue - Homo sapiens, 1218
                aa. [WO200210436-A2,
                07-FEB-2002]
AAY59597          ***Human***   Serrate protein         1 . . . 1218  1215/1218
       (99%)        0.0
                sequence - Homo sapiens,        1 . . . 1218  1216/1218.  .  .
DETD   .  .  .  1)     1 . . . 1218 1218/1218 (100%)     0.0
                (hJ1) - Homo sapiens            1 . . . 1218 1218/1218 (100%)
                (  ***Human***  ), 1218 aa.
Q9QXX0          Jagged 1 precursor (Jagged 1) -  1 . . . 1218 1176/1218 (96%)
       0.0
                Mus musculus (Mouse),           1.  .  .
DETD   .  .  .  for NOV18a
                                                NOV18a        Identities/
                                                Residues/     Similarities for
Geneseq         Protein/Organism/Length         Match         the Matched
Identifier      [Patent #, Date]                Residues      Region
       Expect Value

ABG23422        Novel   ***human***   diagnostic        8 . . . 153  123/153
       (80%)    3e-58
                protein #23413 - Homo           15 . . . 163  127/153 (82%)
                sapiens, 163 aa.
                (WO200175067-A2,
                11-OCT-2001]
AAM79058          ***Human***   protein SEQ ID NO       8 . . . 153  116/146
       (79%)    1e-56
                1720 - Homo sapiens, 141 aa.    2 . . . 141  122/146 (83%)
                [WO200157190-A2,
                09-AUG-2001]
AAY94922          ***Human***   secreted protein clone  8 . . . 153  115/146
       (78%)    1e-55
                pv6_1 protein sequence SEQ      2 . . . 141  121/146 (82%)
                ID NO: 50 - Homo sapiens,
                141 aa. [WO200009552-A1,
                24-FEB-2000]
ABG23423        Novel   ***human***   diagnostic        8 . . . 158  115/151
       (76%)    2e-55
                protein #23414 - Homo           35 . . . 179  122/151 (80%)
```

```
              sapiens, 209 aa.
              [WO200175067-A2,
              11-OCT-2001]
AAM80042        ***Human***   protein SEQ ID NO        8 . . . 141  104/134
       (77%)   3e-47
              3688 - Homo sapiens, 133 aa.  11 ..  .  .
DETD   .  .  .  8 . . . 153  116/146 (79%)    4e-56
           protein              2 . . . 141  122/146 (83%)
           HTMP10 - Homo
           sapiens (  ***Human***  ),
           141 aa.
Q29102     Transmembrane        8 . . . 153  104/147 (70%)    5e-50
           protein sp83.5 -     2 . . . 142  117/147.  .  .
DETD   .  .  .  (91%)    1e-75
              secreted protein  1 . . . 149  142/149 (94%)
              designated
              BMS155 - Homo
              sapiens, 149 aa
              [WO9933979-A2,
              08-JUL-1999]
AAY53042        ***Human***   secreted     1 . . . 149  137/149 (91%)    1e-75
              protein clone     1 . . . 149  142/149 (94%)
              pu282_10 protein
.  .  .  149  137/149 (91%)    1e-75
              108-005-5-0-F6-   1 . . . 149  142/149 (94%)
              FL - Homo
              sapiens, 149 aa.
              [WO9940189-A2,
              12-AUG-1999]
AAY60146        ***Human***               1 . . . 149  137/149 (91%)    1e-75
              endometrium      23 . . . 171  142/149 (94%)
              tumour EST
              encoded protein
.  .  .
DETD   .  .  .  149  137/149 (91%)    4e-75
           protein HSF-28)      1 . . . 149  142/149 (94%)
           (Hypothetical 16.8
           kDa protein) -
           Homo sapiens
           (  ***Human***  ), 149 aa.
Q9P075     HSPC307 - Homo       1 . . . 149  137/149 (91%)    4e-75
           sapiens (  ***Human***  ),     19 . . . 167  142/149 (94%)
           167 aa (fragment).
Q9CPZ2     2310008M10Rik        1 . . . 149  136/149 (91%)    9e-75
           protein.  .  .  aa.
Q9P1R4     HDCMD45P -           1 . . . 149  136/149 (91%)    3e-74
           Homo sapiens        12 . . . 160  141/149 (94%)
           (  ***Human***  ), 160 aa
           (fragment).
Q8TBU1     Similar to DC2      31 . . . 149  118/119 (99%)    4e-63
           protein - Homo       1 . . . 119  118/119 (99%)
           sapiens (  ***Human***  ),
           119 aa.
DETD   .  .  .  NOV20a     Identities/
              Protein/            Residues/  Similarities for
Geneseq       Organism/Length     Match      the Matched      Expect
Identifier    [Patent #, Date]    Residues   Region           Value

AAU84371      Novel  ***human***           1 . . . 225 225/246 (91%)
       e--134
              secreted or         1 . . . 246 225/246 (91%)
              membrane-
              associated protein
              #10 - Homo
              sapiens, 246 aa.
              [WO200204600-A2,
              17-JAN-2002]
AAB88447        ***Human***   membrane      1 . . . 225 225/246 (91%)
       e--134
              or secretory        1 . . . 246 225/246 (91%)
              protein clone
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. . . 225 225/246 (91%)    e--134
              designated          1 . . . 246 225/246 (91%)
              PRO1384 - Homo
              sapiens, 246 aa.
              [WO200056889-A2,
              28-SEP-2000]
AAB29580        ***Human***   adipocyte      1 . . . 225 225/246 (91%)
       e--134
              complement related   1 . . . 246 225/246 (91%)
              protein homolog
              zacrp3, SEQ ID
              NO:2 - Homo
              sapiens, 246
              aa.
              [WO200063377-A1,
              26-OCT-2000]
AAB15548        ***Human***   immune         1 . . . 225 225/246 (91%)
       e--134
              system molecule      1 . . . 246 225/246 (91%)
              from Incyte. . .
DETD  . . . e-134
              tumor necrosis     1 . . . 246  225/246 (91%)
              factor-related
              protein 3
              precursor
              (Secretory
              protein
              CORS26) -
              Homo sapiens
              (  ***Human***  ), 246 aa.
Q9ES30        Collagenous        1 . . . 225  215/246 (87%)    e-127
              repeat-containing  1 . . . 246  217/246 (87%)
              sequence. . . . . . 126  98/120 (81%)    2e-53
              from Patent       101 . . . 220  99/120 (81%)
              WO0149728 -
              Homo sapiens
              (  ***Human***  ), 223 aa.
Q9ESN4        Gliacolin          45 . . . 222  66/194 (34%)    1e-22
              precursor - Mus    64 . . . 253  97/194 (49%)
              musculus
              (Mouse), 255 aa.
Q8TE71        EEGIL - Homo       88 . . . 223  51/138 (36%)    3e-22
              sapiens (  ***Human***  ),  940 . . .      87/138 (62%)
              1077 aa.          1076
DETD  . . . . 428         377/379 (99%)      0.0
              Homo sapiens, 379 aa.         1 . . . 379          377/379 (99%)
              [US5691460-A, 25 NOV.
              1997]
AAR51108        ***Human***   epidermal surface       50 . . . 326
       276/277 (99%)      e-148
              antigen - Homo sapiens, 291   1 . . . 277. . .
DETD  . . . Mus
              musculus (Mouse), 428 aa.
Q9BTI6        Similar to flotillin 2 - Homo 1 . . . 375   374/375 (99%)
       0.0
              sapiens (  ***Human***  ), 385 aa.      1 . . . 375   374/375
       (99%)
Q14254        Flotillin-2 (Epidermal surface 50 . . . 428   379/379 (100%)
       0.0
              antigen) (ESA) - Homo         1 . . . 379    379/379 (100%)
              sapiens (  ***Human***  ), 379 aa.
Q60634        Flotillin-2 (Epidermal surface 50 . . . 428  376/379 (99%)
       0.0
              antigen) (ESA) - Mus          1 . .. . .
DETD  . . . Results for NOV22a
                                            NOV22a      Identities/
                                            Residues/   Similarities
Geneseq       Protein/Organism/Length       Match       for the Matched
       Expect
Identifier    [Patent #, Date]              Residues    Region            Value
```

AAU76337 ***Human*** anti-dual integrin 1 . . . 260 260/260 (100%) e-153
protein #3 - Homo sapiens, 1 . . . 260 260/260 (100%)
799 aa. [WO200212501-A2,
14 FEB. 2002]
AAW02194 ***Human*** integrin beta subunit 1 . . . 260 260/260 (100%) e-153
protein, beta-5 - Homo 1 . . . 260. . . 149/260 (57%)
5e-77
(truncated) - Mus sp, 720 aa. 6 . . . 257 186/260 (71%)
[WO9708316-A1, 06 MAR.
1997]
AAU76336 ***Human*** anti-dual integrin 5 . . . 259 149/260 (57%)
1e-76
protein #2 - Homo sapiens, 7 . . . 258. . .
DETD . . . the Matched Expect

Number	Protein/Organism/Length	Residues	Portion	Value
A38308	integrin beta-5 chain	1 . . . 260	260/260 (100%)	e-153
	precursor - ***human*** , 799 aa.	1 . . . 260	260/260 (100%)	
P18084	Integrin beta-5 precursor -	1 . . . 260	260/260 (100%)	e-153
	Homo sapiens (***Human***), 799 aa.	1 . . . 260	260/260 (100%)	
O70309	Integrin beta-5 precursor -	1 . . . 260	241/260 (92%). . .	

DETD . . . Results for NOV23a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV23a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABB08207	***Human*** type II Interleukin-1	1 . . . 377	375/398 (94%)	0.0
	receptor - Homo sapiens, 398	1 . . . 398	376/398 (94%)	
	aa. [WO200187328-A2, 22 NOV. 2001]			
AAE16581	***Human*** interleukin-1 receptor	1 . . . 377	375/398 (94%)	0.0
	DNAX designation 2 (IL-1RD2) protein - Homo sapiens, 398 aa. [US6326472-B1, 04 DEC. 2001]	1 . . . 398	376/398 (94%)	
AAU78089	***Human*** interleukin 1R2 (IL-1R2) protein sequence - Homo sapiens, 398 aa. [WO200211767-A2, 14 FEB. 2002]	1 . . . 377	375/398 (94%)	0.0
		1 . . . 398	376/398 (94%)	
AAM24185	***Human*** EST encoded protein	1 . . . 377	375/398 (94%)	0.0
	SEQ ID NO: 1710 - Homo sapiens, 398 aa. [WO200154477-A2, 02 AUG. 2001]	1 . . . 398	376/398 (94%)	
AAB37792	***Human*** interleukin-1	1 . . . 377	375/398 (94%)	0.0
	receptor, type II precursor -	1 . . . 398	376/398. . .	
DETD . . .	377 375/398 (94%) 0.0			
	precursor (IL-1R-2) (IL-1R-beta) (Antigen CDw121b) - Homo sapiens (***Human***), 398 aa.	1 . . . 398	376/398 (94%)	
Q29612	Interleukin-1 receptor, type II	1 . . . 372	342/393 (87%)	0.0
	precursor (IL-1R-2) (IL-1R-	1	interleukin-1	1 . .

. 275 273/296 (92%) e-159
receptor - Homo sapiens 1 . . . 296 274/296 (92%)
(***Human***), 296 aa.
Q9N2H5 Interleukin-1 receptor type II 4 . . . 376 258/394 (65%)
e-147
precursor - Equus caballus 4 .. . .
DETD . . . Results for NOV24a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV24a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABB04610	***Human*** quinoprotein dehydrogenase 33 protein SEQ ID NO: 2 - Homo sapiens, 302 aa. [CN1307126-A, 08 AUG. 2001]	1 . . . 284 1 . . . 284	283/284 283/284 (99%)	(99%) e-160
ABB05665	***Human*** transmembrane protein clone amy2.sub.--11d2 #2 - Homo sapiens, 552 aa. [WO200198454-A2, 27 DEC. 2001]	61 . . . 615 6 . . . 548	146/565 261/565 (45%)	(25%) 3e-46
ABB89951	***Human*** polypeptide SEQ ID NO 2327 - Homo sapiens, 552 aa. [WO200190304-A2, 29 NOV. 2001]	61 . . . 615 6 . . . 548	145/565 260/565 (45%)	(25%) 1e-45
ABB89787	***Human*** polypeptide SEQ ID NO 2163 - Homo sapiens,	232 . . . 324 1	83/99	(83%) 3e-39
DETD	. . . . . 624 aa.	580/624 (92%)		
Q9P261	KIAA1467 protein - Homo sapiens (***Human***), 432 aa (fragment).	191 . . . 622 1 . . . 432	432/432 (100%) 432/432	0.0 (100%)
Q99L10	Similar to RIKEN cDNA protein - Homo sapiens (***Human***), 636 aa.	440 . . . 622 72 . . . 605	152/183. . . kDa 261/558 (45%)	61 . . . 615 145/558 (25%) 1e-46
Q9HOX4	Hypothetical 59.7 kDa protein - Homo sapiens (***Human***), 552 aa.	61 . . . 615 6 . . . 548	146/565 (25%) 261/565 (45%)	8e-46
DETD	. . . clone HP10508 protein sequence - Homo sapiens, 231 aa. [WO200000506-A2, 06 JAN. 2000]	1 . . . 231	231/257 (89%)	
ABB90256	***Human*** polypeptide SEQ ID NO 2632 - Homo sapiens, 240 aa. [WO200190304-A2, 29 NOV. 2001]	1 . . . 232 1 . . . 206	205/232 206/232 (88%)	(88%) e-111
AAU83615	***Human*** PRO protein, Seq ID No 48 - Homo sapiens, 222 aa. [WO200208288-A2, 31 JAN. 2002]	19 . . . 232 1 . . . 188	187/214 188/214 (87%)	(87%) 1e-99
AAG81326	***Human*** AFP protein sequence SEQ ID NO: 170 - Homo sapiens, 222 aa. [WO200129221-A2, 26 APR. 2001]	19 . . . 232 1 . . . 188	187/214 188/214 (87%)	(87%) 1e-99

AAB43588 ***Human*** cancer associated 102 . . . 232 127/131 (96%) 9e-70
protein sequence SEQ ID 79 . . . 209 129/131. . .
DETD . . . Patent 19 . . . 232 187/214 (87%) 4e-99
WO0129221 - Homo sapiens 1 . . . 188 188/214 (87%)
(***Human***), 222 aa.
Q9D817 2010001C09Rik protein - 1 . . . 232 163/232 (70%) 3e-82
Mus musculus (Mouse), 223 10 . . . . . 6e-26
protein (Similar to RIKEN 17 . . . 177 104/193 (53%)
cDNA 1810017F10 gene)
(Beta-casein-like protein) -
Homo sapiens (***Human***), 222
aa.
Q8VCL0 RIKEN cDNA 1810017F10 18 . . . 210 69/195 (35%) 1e-24
gene - Mus musculus 17 . . . . .
DETD . . . 81/94 (86%) 8e-37
inhibitor, clade G (C1 17 . . . 109 83/94 (88%)
inhibitor), member 1 - Homo
sapiens (***Human***), 500 aa.
P05155 Plasma protease C1 inhibitor 1 . . . 94 81/94 (86%) 8e-37
precursor (C1 Inh) (C1 Inh) - 17 . . . 109 83/94 (88%)
Homo sapiens (***Human***), 500
aa.
Q95J12 Complement C1 inhibitor - Pan- 2 . . . 82 75/81 (92%) 3e-34
troglodytes (Chimpanzee), 162 1 . . . 80 77/81 (94%)
aa (fragment).
Q16304 C1-inhibitor - Homo sapiens 76 . . . 145 67/70 (95%) 7e-32
(***Human***), 83 aa (fragment). 14 . . . 83 68/70 (96%)
P97290 Plasma protease C1 inhibitor 76 . . . 144 57/69. . .
DETD . . . Results for NOV27a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV27a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU81226 (93%)	***Human*** lung cancer protein, 0.0	1 . . . 416	391/416	
	Seq ID No 62 - Homo	1	polypeptide SEQ ID	
NO	64 . . . 371	153/316 (47%)		
	7500 - Drosophila melanogaster, 373 aa. [WO200171042-A2, 27-SEP-2001]			
AAB88597 (23%)	***Human*** hydrophobic domain 7e-14	8 . . . 322	74/315	
	containing protein clone HP03670 #121 - Homo sapiens, 337 aa. [WO200112660-A2, 22-FEB-2001]	24 . . . 329	137/315 (43%)	
AAB56473 (23%)	***Human*** prostate cancer 1e-13	8 . . . 322	74/315	
	antigen protein sequence	28 . . . 333	136/315 (42%)	

. . .
DETD 309 80/295 (27%) 1e-16
acetylgalactosamine 49 . . . 341 132/295 (44%)
transporter (UDP-
GlcA/UDP-GalNAc
transporter) - Homo sapiens
(***Human***), 355 aa.
DETD . . . Results for NOV28a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV28a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAR13490 (91%)	***Human*** C4 binding protein - e-113	13 . . . 218	190/208	
	Homo sapiens, 581 aa.	1 . . . 208	193/208 (92%)	

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            [WO9111461-A, 08-AUG-
            1991]
AAB57162      ***Human***   prostate cancer        62 . . . 170  107/109
      (98%)   1e-61
            antigen protein sequence      1 . . . 109  108/109 (98%)
. . .  sperm protein           1 . . . 192  132/193 (68%)
            designated sp56 - Mus sp,
            579 aa. [WO9800440-A1,
            08-JAN-1998]
AAG68150    Codon modified   ***human***   DAF      32 . . . 217   74/191
      (38%)   3e-32
            protein sequence SEQ ID       22 . . . 212  106/191 (54%)
. . .
DETD  . . .  218  202/220 (91%)    e-120
            chain precursor (C4bp)        5 . . . 224  205/220 (92%)
            (Proline-rich protein) (PRP) -
            Homo sapiens (  ***Human***  ), 597
            aa.
Q28065      C4b-binding protein alpha      1 . . . 211  127/214 (59%)   5e-71
            chain precursor (C4bp) - Bos   5 .. . .
DETD  . . .  for NOV29a

                                          NOV29a        Identities/
                                          Residues/     Similarities for
Geneseq     Protein/Organism/Length       Match         the Matched
      Expect
Identifier  [Patent #, Date]              Residues      Region          Value

AAB07747    A   ***human***   cancer-associated      33 . . . 319 148/287
      (51%)   7e-89
            protein-1 (CAP-1) - Homo      178 . . . 464 205/287 (70%)
. . .
DETD  . . . . . 321 289/289 (100%)      e-168
            protein SRPX precursor -      176 . . . 464 289/289 (100%)
            Homo sapiens (  ***Human***  ), 464
            aa.
Q63769      Sushi repeat-containing        33 . . . 321 279/289 (96%)
      e-164
            protein SRPX precursor        176 . . . 464.  . .
DETD  . . .  sequence SEQ ID        65 . . . 483 417/419 (99%)
            NO: 82 - Homo sapiens, 546
            aa. [WO200110902-A2, 15-
            FEB-2001]
AAB70547      ***Human***   PRO17 protein            32 . . . 450 417/419
      (99%)   0.0
            sequence SEQ ID NO: 34 -      101 . . . 519 417/419 (99%)
            Homo sapiens, 582 aa.
            [WO200110902-A2, 15-
            FEB-2001]
AAB80265      ***Human***   PRO334 protein -         36 . . . 450 383/415
      (92%)   0.0
            Homo sapiens, 509 aa.          88 . . . 473 383/415 (92%)
            [WO200104311-A1, 18-
            JAN-2001]
AAU29049      ***Human***   PRO polypeptide          36 . . . 450 383/415
      (92%)   0.0
            sequence #26 - Homo            88 . . . 473 383/415.  . .
DETD  . . .  Patent        32 . . . 450 417/419 (99%)   0.0
            WO0110902 - Homo sapiens      101 . . . 519 417/419 (99%)
            (  ***Human***  ), 582 aa.
Q91V88      POEM (NEPHRONECTIN             36 . . . 450 363/416 (87%)   0.0
            short isoform) - Mus           88 . . ..  .  .
DETD  . . .  Results for NOV31a

                                          NOV31a        Identities/
                                          Residues/     Similarities for
Geneseq     Protein/Organism/Length       Match         the Matched
      Expect
Identifier  [Patent #, Date]              Residues      Region
      Value

AAB70544      ***Human***   PRO14 protein            1 . . . 840  840/840
      (100%)    0.0
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            sequence SEQ ID NO: 28 -       1 . . . 840  840/840 (100%)
            Homo sapiens, 840 aa.
            [WO200110902-A2, 15-
            FEB-2001]
AAO20441    Protein of the   ***human***   cancer    1 . . . 840  840/859
      (97%)      0.0
            suppressor gene 98 - Homo     36 . . . 894  840/859 (97%)
            sapiens, 894 aa.
            [CN1328030-A, 26-DEC-
            2001]
AAU14316      ***Human***   novel protein #187 -     1 . . . 840  840/859
      (97%)      0.0
            Homo sapiens, 859 aa.          1 . . . 859  840/859 (97%)
            [WO200155437-A2, 02-
            AUG-2001]
AAB42317      ***Human***   ORFX ORF2081             1 . . . 840  840/859
      (97%)      0.0
            polypeptide sequence SEQ       1 . . . 859  840/859 (97%)
. . .
DETD  . . .  Patent        1 . . . 840  840/840 (100%)     0.0
            WO0110902 - Homo sapiens       1 . . . 840  840/840 (100%)
            (  ***Human***  ), 840 aa.
Q9Y561      ST7 protein - Homo sapiens     1 . . . 840  840/859 (97%)
      0.0
            (  ***Human***  ), 859 aa.               1 . . . 859  840/859
      (97%)
Q9BE74      Hypothetical 73.8 kDa          169 . . . 840 663/672 (98%)
      0.0
. . . Patent        1 . . . 423  422/423 (99%)      0.0
            WO0119856 - Homo sapiens       1 . . . 423  422/423 (99%)
            (  ***Human***  ), 430 aa.
CAC33423    Sequence 29 from Patent        1 . . . 423  422/442 (95%)
      0.0
            WO0110902 - Homo sapiens       1 . . . 442  422/442 (95%)
            (  ***Human***  ), 449 aa.
DETD  . . .  Results for NOV32a
                                           NOV32a
                                           Residues/     Identities/
Geneseq     Protein/Organism/Length        Match         Similarities for the
      Expect
Identifier  [Patent #, Date]               Residues      Matched Region
      Value

AAB42154      ***Human***   ORFX ORF1918             1 . . . 461  461/461
      (100%)     0.0
            polypeptide sequence SEQ      19 . . . 479  461/461 (100%)
            ID NO: 3836 - Homo sapiens,
            479 aa. [WO200058473-A2,
            05-OCT-2000]
AAM41619      ***Human***   polypeptide SEQ ID       7 . . . 461  454/455
      (99%)      0.0
            NO 6550 - Homo sapiens,        3 . . . 457  454/455 (99%)
            457 aa. [WO200153312-A1,
            26-JUL-2001]
AAE06600      ***Human***   protein having           20 . . . 461  442/442
      (100%)     0.0
            hydrophobic domain,            1 . . . 442  442/442 (100%)
            HP10787 - Homo sapiens,
            442 aa. [WO200149728-A2,
            12-JUL-2001]
AAM39833      ***Human***   polypeptide SEQ ID       20 . . . 461  439/442
      (99%)      0.0
            NO 2978 - Homo sapiens,        1 . . . 442  439/442 (99%)
            442 aa. [WO200153312-A1,
            26-JUL-2001]
AAY12280      ***Human***   5' EST secreted          20 . . . 124  104/105
      (99%)      3e-54
            protein SEQ ID NO: 311 -       1 . . .  .  .
DETD  . . .  the Expect
Number      Protein/Organism/Length        Residues      Matched Portion
      Value
```

CAD39027	Hypothetical protein - Homo	6 . . . 461	456/456 (100%)
0.0			
	sapiens (***Human***), 456 aa	1 . . . 456	456/456
(100%)			
	(fragment).		
Q9BX97	PV1 protein - Homo sapiens	20 . . . 461	442/442 (100%)
0.0			
	(***Human***), 442 aa.	1 . . . 442	442/442
(100%)			
Q9BZD5	Fenestrated-endothelial	20 . . . 461	441/442 (99%)
0.0			
	linked structure protein - Homo sapiens (***Human***), 442 aa.	1 . . . 442	441/442 (99%)
BAC04681	CDNA FLJ38711 fis, clone	20 . . . 461	436/442 (98%)
0.0			
	KIDNE2003507, highly similar to Homo sapiens PV1 protein (PLVAP) mRNA - Homo sapiens (***Human***), 437 aa.	1 . . . 437	436/442 (98%)
Q91VC4	MECA32 (Similar to	20 . . . 461	273/442 (61%)
e-156			
	PLASMALEMMA vesicle	1 . . . 438. . .	
DETD . . .	Results for NOV33a		

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV33a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB70543	***Human*** PRO13 protein	1 . . . 261	261/261 (100%)	e-154
	sequence SEQ ID NO: 26 - Homo sapiens, 261 aa. [WO200110902-A2, 15-FEB-2001]	1 . . . 261	261/261 (100%)	
AAE15853	***Human*** SEZ6 protein - Homo	1 . . . 242	242/242 (100%)	e-140
	sapiens, 853 aa. [WO200183552-A2, 08-NOV-2001]	1 . . . 242	242/242 (100%)	
AAU81976	***Human*** secreted protein	1 . . . 242	242/242 (100%)	e-140
	SECP2 - Homo sapiens, 994 aa. [WO200198353-A2, 27-DEC-2001]	1 . . . 242	242/242 (100%)	
AAB70542	***Human*** PRO12 protein	1 . . . 242	242/242 (100%)	e-140
	sequence SEQ ID NO: 24 - Homo sapiens, 526 aa. [WO200110902-A2, 15-FEB-2001]	1 . . . 242	242/242 (100%)	
AAB70541	***Human*** PRO11 protein	1 . . . 242	242/242 (100%)	e-140
	sequence SEQ ID NO: 22 -	1		
DETD . . .	Patent 1 . . . 261 261/261 (100%) e-154			
	WO0110902 - Homo sapiens (***Human***), 261 aa.	1 . . . 261	261/261 (100%)	
CAC33420	Sequence 23 from Patent	1 . . . 242	242/242 (100%)	e-140
	WO0110902 - Homo sapiens (***Human***), 526 aa.	1 . . . 242	242/242 (100%)	
CAC33418	Sequence 19 from Patent	1 . . . 242	242/242 (100%)	e-140
	WO0110902 - Homo sapiens (***Human***), 525 aa.	1 . . . 242	242/242 (100%)	
CAC33417	Sequence 17 from Patent	1 . . . 242	242/242 (100%)	e-140

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            WO0110902 - Homo sapiens      1 . . . 242 242/242 (100%)
            (  ***Human***  ), 525 aa.
CAC33416    Sequence 15 from Patent       1 . . . 242 242/242 (100%)
      e-140
            WO0110902 - Homo sapiens      1 . . . 242 242/242 (100%)
            (  ***Human***  ), 994 aa.
DETD  .  .  .  Results for NOV34a
                                          NOV34a
                                          Residues/     Identities/
Geneseq     Protein/Organism/Length       Match         Similarities for the
      Expect
Identifier  [Patent #, Date]              Residues      Matched Region
      Value

AAB54163      ***Human***  pancreatic cancer       1 . . . 112  112/112
      (100%)      3e-62
            antigen protein sequence      20 . . . 131  112/112 (100%)
            SEQ ID NO: 615 - Homo
            sapiens, 131 aa.
            [WO200055320-A1, 21
            SEP. 2000]
AAY91513      ***Human***  secreted protein        28 . . . 111  28/84 (33%)
      1e-07
            sequence encoded by gene 63   33 . . . 114  38/84 (44%)
            SEQ ID NO: 186 - Homo
            sapiens, 122 aa.
            [WO200006698-A1, 10
            FEB. 2000]
AAY35930    Extended  ***human***  secreted       28 . . . 111  28/84 (33%)
      1e-07
            protein sequence, SEQ ID      33 . . . 114  38/84 (44%)
            NO. 179 - Homo sapiens,
            121 aa. [WO9931236-A2,
            24 JUN. 1999]
AAB62640      ***Human***  colipase-like protein-  32 . . . 111  26/80 (32%)
      3e-07
            1 (Zclps1) - Homo sapiens,    34 . . . 111  36/80 (44%)
            118 aa. [WO200136466-A2,
            25 MAY 2001]
AAB62648      ***Human***  colipase-like protein-  32 . . . 109  25/78 (32%)
      1e-06
            1 (Zclps1) fragment - Homo    22 . . . 97.  .  .
DETD  .  .  .  the          Expect
Number      Protein/Organism/Length       Residues      Matched Portion
      Value

P04118      Colipase precursor - Homo     1 . . . 112   112/112 (100%)
      9e-62
            sapiens (  ***Human***  ), 112 aa.      1 . . . 112   112/112
      (100%)
P19090      Colipase precursor - Canis    1 . . . 112   88/112 (78%).  .  .
DETD  .  .  .  NOV34b (CG55698-02; SEQ ID NO:180). The arrow indicates the
      signal sequence cleavage site. Since the homology between the porcine
      and  ***human***  lipases is high, the x-ray crystal structure of the
      porcine lipase is a suitable comparison for the effects of NOV34b.  .  .
DETD  .  .  .  YTVTLHGEVRGHSTRPLAVEVVTEDLPQLGDLAVSEVGWDGLRLNWTAADNAYEHFVIQV

                     QEVNKVEAAQNLTLPGSLRAVDIPGLEAATPYRVSIYGVIRGYRTPVLSAEASTAK
      EPEI

                     GNLNVSDITPESFNLSWMATDGIFETFTIEIIDSNRLLETVEYNISGAERTAHISG
      LPPS

                     TDFIVYLSGLAPSIRTKTISATATTEAEPEVDNLLVSDATPDGFRLSWTADECVFD
      NFVL

                     KIRDTKKQSEPLEITLLAPERTRDITCLREATEYEIELYGISKGRRSQTVSAIATT
      AMGS

                     PKEVIFSDITENSATVSWRAPTAQVESFRITYVPITGGTPSMVTVDGTKTQTRLVK
      LIPG
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VEYLVSIIAMKGFEESEPVSGSFTTALDGPSGLVTANITDSEALARWQPAIATVDS
YVIS

YTGEKVPEITRTVSGNTVEYALTDLEPATEYTLRIFAEKGPQKSSTITAKFTTDLD
SPRD

LTATEVQSETALLTWRPPRASVTGYLLVYESVDGTVKEVIVGPDTTSYSLADLSPS
THYT

AKIQALNGPLRSNMIQTIFTTIGLLYPFPKDCSQAMLNGDTTSGLYTIYLNGDKAQ
ALEV

FCDMTSDGGGWIVFLRRKNGRENFYQNWKAYAAGFGDRREEFWLGLDNLNK
*** ITAQGQYEL***

RVDLRDHGETAFAVYDKFSVGDAKTRYKLKVEGYSGTAGDSMAYHNGRSFSTFDKD
TDSA

ITNCALSTRGFWYRNCHRVNLMGRYGDNNHSQGVNWFHWKGHEHSIQFAEMKLRPS
NFRN

LEGRRKRA

SEQ ID NO: 185 5375 bp
NOV35c, GAATTCGCTAGAGCCCTAGAGCCCCAGCAGCACCCAGCCAAACCCACCTCCACCAT
GGGG
CG55832-02
DNA Sequence GCCATGACTCAGCTGTTGGCAGGTGTCTTTCTTGCTTTCCTPGCCCTCGCTACCGA
AGGT

GGGGTCCTCAAGAAAGTCATCCGGCACAAGCGACACAGTGGGGTGAACGCCACCCT
GCCA

. . .

DETD . . . Results for NOV35a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV35a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAR94562	***Human*** cytotactin - Homo sapiens, 2199 aa. [WO9608513-A1, 21 MAR. 1996]	1 . . . 2199	2199/2199 (100%)	0.0
AAB36935	***Human*** tenascin-C - Homo sapiens, 2201 aa. [WO9608513-A1, 21 MAR. 1996]	1 . . . 2201	2198/2201. . . . 1602 (99%)	0.0
AAM39043	***Human*** polypeptide SEQ ID NO 2188 - Homo sapiens, 4618 aa. [WO200153312-A1, 26 JUL. 2001]	627 . . . 2194 2901 . . . 4616	544/1741 (31%) 834/1741 (47%)	0.0
AAW18824	***Human*** restrictin - Homo sapiens, 1358 aa. [WO9608513-A1, 21 MAR. 1996]	484 . . . 1414 188 . . . 1107	338/935 (36%) 528/935. .	0.0

DETD 2201 2198/2201 (99%)
(Neuronectin) (GMEM) (JI)
(Miotendinous antigen)
(Glioma-associated-
extracellular matrix antigen)
(GP 150-225) (Tenascin-C)

(TN-C) - Homo sapiens
(***Human***), 2201 aa.
JQ1322 tenascin precursor - mouse, 1 . . . 1796 1282/1807 (70%)
0.0
2019 aa. 1 . . . . . .
DETD 1137 1137/1137 (100%) 0.0
Homo sapiens, 1137 aa. 1 . . . 1137 1137/1137 (100%)
[WO200066628-A1,
09-NOV-2000]
AAU29083 ***Human*** PRO polypeptide 1 . . . 1137 1109/1147
(96%) 0.0
sequence#60 - Homo 1 . . . 1141 1113/1147 (96%)
sapiens, 1141 aa.
[WO200168848-A2,
20-SEP-2001]
AAB44308 ***Human*** PRO768 (UNQ406) 1 . . . 1137 1109/1147
(96%) 0.0
protein sequence SEQ ID 1 . . . 1141 1113/1147 (96%)
NO: 437 - Homo sapiens,
1141 aa. [WO200053756-
A2, 14-SEP-2000]
AAY41752 ***Human*** PRO768 protein 1 . . . 1137 1109/1147
(96%) 0.0
sequence - Homo sapiens, 1 . . . 1141 1113/1147 (96%)
1141 aa. [WO9946281-A2,
16-SEP-1999]
AAB94058 ***Human*** protein sequence 159 . . . 1137 970/979
(99%) 0.0
SEQ ID NO: 14232 - Homo 1 . . . . . .
DETD . . . the Expect
Number Protein/Organism/Length Residues Matched Portion
Value

JC5950 integrin alpha-7 chain 1 . . . 1137 1137/1137 (100%)
0.0
precursor - ***human*** , 1137 aa. 1 . . . 1137 1137/1137
(100%)
Q13683 Integrin alpha-7 precursor -- 1 . . . 1137 1137/1181 (96%)
0.0
Homo sapiens (***Human***) 1 . . . 1181 1137/1181
(96%)
1181 aa.
I61186 alpha-7 integrin - mouse, 14 . . . 1137 985/1124 (87%). . .
DETD . . .
TABLE 37C

Geneseq Results for NOV37a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	Residues/ Match Residues	Similarities for the Matched Region	Expect Value
AAM39605	***Human*** polypeptide SEQ ID NO 2750 - Homo sapiens, 515 aa. [WO200153312-A1, 26-JUL-2001]	878 . . . 1332 1 . . . 456	455/456 455/456 (99%)	(99%) 0.0
AAM41391	***Human*** polypeptide SEQ ID NO 6322 - Homo sapiens, polypeptide SEQ ID NO 2988 - Drosophila melanogaster, 1523 aa. [WO200171042-A2, 27-SEP-2001]	1072 . . . 1332 1 . . . . . . 1 . . . 660	261/262 e-141 412/705 (57%)	(99%) e-147
AAB43113	***Human*** ORFX ORF2877 polypeptide sequence SEQ ID NO: 5754 - Homo	1171 . . . 1332 1 . . . 162	162/162 162/162 (100%)	(100%) 3e-87

sapiens, 221 aa.
[WO200058473-A2,
05-OCT-2000]

AAB41768 ***Human*** ORFX ORF1532 683 . . . 801 115/120 (95%) 6e-59
polypeptide sequence SEQ 2 . . . 121 116/120 (95%)

. . .

DETD . . . the Expect

Number	Protein/Organism/Length	Residues	Matched Portion	Value
Q9H3X8	DJ927M24.2 (KIAA1219) -	1 . . . 1169	1143/1208 (94%)	0.0
	Homo sapiens (***Human***), 1188 aa (fragment).	1 . . . 1188	1144/1208 (94%)	
BAA86533	KIAA1219 protein - Homo	651 . . . 1332	674/683 (98%)	0.0
	sapiens (***Human***), 1112 aa (fragment).	371 . . . 1053	677/683 (98%)	
CAD39096	Hypothetical protein - Homo	651 . . . 1332	674/684 (98%)	0.0
	sapiens (***Human***), 1333 aa (fragment).	591 . . . 1274	677/684 (98%)	
Q9ULK1	KIAA1219 protein - Homo	860 . . . 1332	473/473 (100%)	0.0
	sapiens (***Human***), 532 aa (fragment).	1 . . . 473	473/473 (100%)	
Q8WWC0	Hypothetical 47.6 kDa	970 . . . 1332	363/363 (100%)	0.0
	protein - Homo sapiens (***Human***), 423 aa (fragment).	2 . . . 364	363/363 (100%)	

DETD . . . Results for NOV38a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV38a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU12271	***Human*** PRO6094	1 . . . 1013	1013/1023 (99%)	0.0
	polypeptide sequence - Homo sapiens, 1023 aa. [WO200140466-A2, 07 JUN. 2001]	1 . . . 1023	1013/1023 (99%)	
ABG22405	Novel ***human*** diagnostic protein #22396 - Homo sapiens, 990 aa. [WO200175067-A2, 11 OCT. 2001]	29 . . . 1013 6 . . . 990	983/985 (99%) 984/985 (99%)	0.0
ABG05922	Novel ***human*** diagnostic protein #5913 - Homo sapiens, 990 aa. [WO200175067-A2, 11 OCT. 2001]	29 . . . 1013 6 . . . 990	983/985 (99%) 984/985 (99%)	0.0
ABG01221	Novel ***human*** diagnostic protein #1212 - Homo sapiens, 982 aa. [WO200175067-A2, 11 OCT. 2001]	33 . . . 1013 2 . . . 982	981/981 981/981 (100%)	(100%) 0.0
ABG22407	Novel ***human*** diagnostic protein #22398 - Homo	29 . . . 1008 6 . . . 996	967/991 (97%) 971/991 (97%)	0.0

. . .

DETD . . . 1 . . . 1013 1013/1024 (98%) 0.0
precursor - Homo sapiens 1 . . . 1024 1013/1024 (98%)
(***Human***), 1024 aa.
Q9Y2E1 KIAA0927 protein - Homo 1 . . . 872 821/876 (93%)
0.0
sapiens (***Human***), 1001 aa 53 . . . 925 834/876
(94%)
(fragment).
Q9Y3J6 Hypothetical 87.6 kDa 228 . . . 1008 778/791 (98%). . .
protein (DJ268D13.1.2) 1 . . . 791 780/791 (98%)
(seizure related gene 6
(mouse)-like (KIAA0927)
(isoform 2)) - Homo sapiens
(***Human***), 792 aa.
Q9NUI3 DJ268D13.1.3 (Seizure 228 . . . 1004 775/779 (99%)
0.0
related gene 6 (Mouse)-like 1 . . . 777 775/779 (99%)
(KIAA0927) (Isoform 3)) -
Homo sapiens (***Human***), 777
aa (fragment).
O95917 Hypothetical 79.0 kDa 228 . . . 868 641/641 (100%)
0.0
protein (DJ268D13.1.1) 1 . . . 641 641/641 (100%)
(seizure related gene 6
(mouse)-like (KIAA0927)
(isoform 1)) - Homo sapiens
(***Human***), 716 aa.
DETD . . . Results for NOV39a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV39a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB74705	***Human*** membrane associated protein MEMAP-11 - Homo sapiens, 620 aa. [WO200112662-A2, 22 FEB. 2001]	1 . . . 614 7 . . . 620	614/614 (100%) 614/614. . .	0.0
AAW84596	Amino acid sequence of the ***human*** Tango-79 protein - Homo sapiens, 614 aa. [WO9906427-A1, 11 FEB. 1999]	1 . . . 614 1 . . . 614	612/614 (99%) 612/614 (99%)	0.0
AAB80225	***Human*** PRO227 protein - Homo sapiens, 620 aa. [WO200104311-A1, 18 JAN. 2001]	1 . . . 614 7 . . . 620	612/614 (99%) 612/614 (99%)	0.0
AAU12333	***Human*** PRO227 polypeptide sequence - Homo sapiens,	1 . . . 614 7 . . . 620	612/614 (99%) 612/614. . .	0.0
DETD . . .	1 . . . 614 614/614 (100%) 0.0			
	MGC: 17422) - Homo sapiens (***Human***), 614 aa.	1 . . . 614	614/614 (100%)	
Q9N008	Hypothetical 69.2 kDa protein - Macaca fascicularis 1 . .. . . sequence - Mus musculus (Mouse), 614 aa.	1 . . . 614	612/614 (99%)	0.0
CAD38935	Hypothetical protein - Homo sapiens (***Human***), 577 aa (fragment).	38 . . . 614 1 . . . 577	577/577 (100%) 577/577 (100%)	0.0
Q9BZ20	BA438B23.1 (Neuronal	14 . . . 614	365/603 (60%)	0.0
. . . .	606		468/603 (77%)	

(CDNA FLJ31810 fis, clone NT2RI2009289, weakly similar to carboxypeptidase N 83 kDa chain) - Homo sapiens (***Human***), 606 aa.

DETD . . . Results for NOV40a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV40a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU78086	***Human*** CD30-ligand	1 . . . 234	234/234 (100%)	e-135
	(CD30L) protein sequence - Homo sapiens, 234 aa. [WO200211767-A2, 14 FEB. 2002]	1 . . . 234	234/234 (100%)	
AAR45009	Sequence encoded by a ***human*** CD30-L cDNA clone encoding additional N-terminal amino acids - Homo sapiens, 234 aa. [WO9324135-A, 09 DEC. 1993]	1 . . . 234	234/234 (100%)	e-135
		1 . . . 234	234/234 (100%)	
AAR45007	Sequence encoded by a ***human*** CD30-L cDNA clone - Homo sapiens, 215 aa. [WO9324135-A, 09 DEC. 1993]	20 . . . 234	215/215 (100%)	e-123
		1 . . . 215	215/215 (100%)	
AAU78087	Mouse. . .			
DETD	. . . e-134			
	superfamily member 8 (CD30 ligand) (CD30- L) (CD153 antigen) - Homo sapiens (***Human***), 234 aa.	1 . . . 234	234/234 (100%)	
P32972	Tumor necrosis factor ligand superfamily member 8	1 . . . 234	167/240 (69%)	1e-91
		1		

DETD . . . expression analysis by transcript profiling coupled to a gene database query" Nature Biotechnology 17:198-803 (1999). cDNA was derived from various ***human*** samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained. . .

DETD [0607] 2. SeqCalling.TM. Technology: cDNA was derived from various ***human*** samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained. . . were evaluated manually and edited for corrections if appropriate. cDNA sequences from all samples were assembled together, sometimes including public ***human*** sequences, using bioinformatic programs to produce a consensus sequence for each assembly. Each assembly is included in CuraGen Corporation's database.. . .

DETD . . . are sequenced. In silico prediction was based on sequences available in CuraGen Corporation's proprietary sequence databases or in the public ***human*** sequence databases, and provided either the full length DNA sequence, or some portion thereof.

DETD [0610] cDNA libraries were derived from various ***human*** samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained. . .

DETD [0611] Gal4-binding domain (Gal4-BD) fusions of a CuraGen Corportion proprietary library of ***human*** sequences was used to screen multiple Gal4-AD fusion cDNA libraries resulting in the selection of yeast hybrid diploids in each. . . were evaluated manually and edited for corrections if appropriate. cDNA sequences from all samples were

assembled together, sometimes including public ***human*** sequences, using bioinformatic programs to produce a consensus sequence for each assembly. Each assembly is included in CuraGen Corporation's database.. . .

DETD . . . or complete the predicted sequence of the cDNA of the invention. Usually multiple clones were sequenced from one or more ***human*** samples to derive the sequences for fragments. Various ***human*** tissue samples from different donors were used for the RACE reaction. The sequences derived from these procedures were included in. . .

DETD . . . the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related ***human*** sequences from other species. These primers were then employed in PCR amplification based on the following pool of ***human*** cDNAs: adrenal gland, bone marrow, brain--amygdala, brain--cerebellum, brain--hippocampus, brain--substantia nigra, brain--thalamus, brain--whole, fetal brain, fetal kidney, fetal liver, fetal lung,. . .

DETD . . . lines), Panel 4 (containing cells and cell lines from normal tissues and cells related to inflammatory conditions), Panel 5D/5I (containing ***human*** tissues and cell lines with an emphasis on metabolic diseases), AI_comprehensive-panel (containing normal tissue and samples from autoimmune/autoinflammatory diseases), Panel. . .

DETD . . . 2.2, 2.3 and 2.4 generally include two control wells and 94 test samples composed of RNA or cDNA isolated from ***human*** tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative ***Human*** Tissue Network (CHTN) or the National Disease Research Initiative (NDRI) or from Ardais or Clinomics. The tissues are derived from ***human*** malignancies and in cases where indicated many malignant tissues have "matched margins" obtained from noncancerous tissue just adjacent to the. . . surgery (designated "NAT", for normal adjacent tissue, in Table RR). In addition, RNA and cDNA samples were obtained from various ***human*** tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissues were ascertained to be. . .

DETD . . . 1.0 plates are comprised of 93 cDNA samples and two controls. Specifically, 81 of these samples are derived from cultured ***human*** cancer cell lines that had been subjected to serum starvation, acidosis and anoxia for different time periods as well as controls for these treatments, 3 samples of ***human*** primary cells, 9 samples of malignant brain cancer (4 medulloblastomas and 5 glioblastomas) and 2 controls. The ***human*** cancer cell lines are obtained from ATCC (American Type Culture Collection) and fall into the following tissue groups: breast cancer,. . . recommended conditions. The treatments used (serum starvation, acidosis and anoxia) have been previously published in the scientific literature. The primary ***human*** cells were obtained from Clonetics (Walkersville, Md.) and were grown in the media and conditions recommended by Clonetics. The malignant. . .

DETD . . . plates for ARDAIS panel v 1.0 generally include 2 control wells and 22 test samples composed of RNA isolated from ***human*** tissue procured by surgeons working in close cooperation with Ardais Corporation. The tissues are derived from ***human*** lung malignancies (lung adenocarcinoma or lung squamous cell carcinoma) and in cases where indicated many malignant samples have "matched margins". . .

DETD . . . 3.1 are comprised of 94 cDNA samples and two control samples. Specifically, 92 of these samples are derived from cultured ***human*** cancer cell lines, 2 samples of ***human*** primary cerebellar tissue and 2 controls. The ***human*** cell lines are generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall. . .

DETD . . . plate (2 control wells, 94 test samples) composed of RNA (Panel 4R) or cDNA (Panels 4D/4. ID) isolated from various ***human*** cell lines or tissues related to inflammatory conditions. Total RNA from control normal tissues such as colon and lung (Stratagene,. . .

DETD . . . dermal fibroblasts, coronary artery smooth muscle cells, small airway epithelium, bronchial epithelium, microvascular dermal endothelial cells, microvascular lung endothelial cells, ***human*** pulmonary aortic endothelial cells, ***human*** umbilical vein

endothelial cells were all purchased from Clonetics (Walkersville, MD) and grown in the media supplied for these cell. . .

DETD . . . blood of employees at CuraGen Corporation, using Ficoll. LAK cells were prepared from these cells by culture in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco/Life Technologies, Rockville, Md.), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and. . . IL-18 at 5-10 ng/ml for 6 hours. In some cases, mononuclear cells were cultured for 4-5 days in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . . cells using Ficoll and mixing the isolated mononuclear cells 1:1 at a final concentration of approximately 2.times.10.sup.6cells/ml in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol (5.5.times.10.sup.-5M) (Gibco), and 10 mM Hepes. . .

DETD . . . Magnet according to the manufacturer's instructions. Monocytes were differentiated into dendritic cells by culture in DMEM 5% fetal calf serum (***FCS***) (Hyclone, Logan, Utah), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10. . . and 5 ng/ml IL-4 for 5-7 days. Macrophages were prepared by culture of monocytes for 5-7 days in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), 10 mM Hepes (Gibco) and 10% AB ***Human*** Serum or MCSF at approximately 50 ng/ml. Monocytes, macrophages and dendritic cells were stimulated for 6 and 12-14 hours with. . .

DETD . . . with the remaining cells being CD45RA CD4 lymphocytes. CD45RA CD4, CD45RO CD4 and CD8 lymphocytes were placed in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . . for 4 days on anti-CD28 and anti-CD3 coated plates and then harvested the cells and expanded them in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . . second activation and after 4 days of the second expansion culture. The isolated NK cells were cultured in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . .

DETD . . . and then passed through a sieve. Tonsil cells were then spun down and resupended at 10.sup.6 cells/ml in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . .

DETD . . . with PBS. Umbilical cord blood CD4 lymphocytes (Poietic Systems, German Town, MD) were cultured at 10.sup.5-10.sup.6 cells/ml in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), 10 mM Hepes (Gibco). . . the activated Th1, Th2 and Tr1 lymphocytes were washed once in DMEM and expanded for 4-7 days in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), 10 mM Hepes (Gibco). . .

DETD . . . the culture of these cells, we used DMEM or RPMI (as recommended by the ATCC), with the addition of 5% ***FCS*** (Hyclone), 100 PM non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), 10 mM Hepes (Gibco).. . . CCD106 and an airway epithelial tumor line NCI-H292 were also obtained from the ATCC. Both were cultured in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . .

DETD . . . plates for AI_comprehensive panel_v1.0 include two control wells and 89 test samples comprised of cDNA isolated from surgical and postmortem ***human*** tissues obtained from the Backus Hospital and Clinomics (Frederick, Md.). Total RNA was extracted from tissue samples from the Backus. . .

DETD [0669] The plates for Panel 5D and 5I include two control wells and a variety of cDNAs isolated from ***human*** tissues and cell lines

with an emphasis on metabolic diseases. Metabolic tissues were obtained from patients enrolled in the Gestational Diabetes study. Cells were obtained during different stages in the differentiation of adipocytes from ***human*** mesenchymal stem cells. ***Human*** pancreatic islets were also obtained.

DETD . . . of Clonetics/BioWhittaker) in triplicate, except for Donor 3U which had only two replicates. Scientists at Clonetics isolated, grew and differentiated ***human*** mesenchymal stem cells (HuMSCs) for CuraGen based on the published protocol found in Mark F. Pittenger, et al., Multilineage Potential of Adult ***Human*** Mesenchymal Stem Cells Science Apr. 2, 1999: 143-147. Clonetics provided Trizol lysates or frozen pellets suitable for mRNA isolation and. . .

DETD [0680] ***Human*** cell lines were generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall. . .

DETD [0690] The plates for Panel CNSD.01 include two control wells and 94 test samples comprised of cDNA isolated from postmortem ***human*** brain tissue obtained from the Harvard Brain Tissue Resource Center. Brains are removed from calvaria of donors between 4 and. . .

DETD [0699] The plates for Panel CNS_Neurodegeneration_V1.0 include two control wells and 47 test samples comprised of cDNA isolated from postmortem ***human*** brain tissue obtained from the Harvard Brain Tissue Resource Center (McLean Hospital) and the ***Human*** Brain and Spinal Fluid Resource Center (VA Greater Los Angeles Healthcare System). Brains are removed from calvaria of donors between. . .

DETD [0741] F. CG137873-02: ***Human*** Fibrinogen Alpha Chain Precursor Protein-Likew Protein

DETD . . . regulatory region(s) that normally repress the expression of this gene in lung tumor cells. Therefore, targeting this gene with a ***human*** monoclonal antibody that results in an inhibition of the activity of this protein, preferably as it relates to its apoptotic/survival. . .

DETD . . . all or part of the initial or extended sequence were identified by BLASTN searches using the relevant sequence to query ***human*** genomic databases. The genomic clones that resulted were selected for further analysis because this identity indicates that these clones contain. . .

DETD . . . identified was manually assembled and then may have been extended using one or more additional sequences taken from CuraGen Corporation's ***human*** SeqCalling database. SeqCalling fragments suitable for inclusion were identified by the CuraTools.TM. program SeqExtend or by identifying SeqCalling fragments mapping. . .

DETD [1517] Expression of CG51117-05 in ***human*** embryonic kidney 293 cells. A 1.6 kb BamHI-XhoI fragment containing the CG5117-05 sequence was subcloned into BamHI-XhoI digested pCEP4/Sec to. . .

CLM What is claimed is:

8. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a ***human*** disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein the therapeutic comprises the polypeptide. . .

18. The method of claim 17, wherein the subject is a ***human*** .

L13 ANSWER 3 OF 24 USPATFULL on STN

AN 2004:51446 USPATFULL

TI Therapeutic polypeptides, nucleic acids encoding same, and methods of use

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PI US 2004038877 A1 20040226
AI US 2002-262839 A1 20021001 (10)
PRAI US 2001-326483P 20011002 (60)
US 2001-327917P 20011009 (60)
US 2001-328029P 20011009 (60)
US 2001-328056P 20011009 (60)
US 2002-381101P 20020516 (60)
US 2002-371972P 20020412 (60)
US 2001-327342P 20011005 (60)
US 2001-328044P 20011009 (60)
US 2001-328849P 20011012 (60)
US 2002-374738P 20020423 (60)
US 2001-329414P 20011015 (60)
US 2001-330142P 20011017 (60)
US 2002-383830P 20020529 (60)
US 2001-341058P 20011022 (60)
US 2002-373805P 20020419 (60)
US 2002-381635P 20020517 (60)
US 2002-371980P 20020412 (60)
US 2001-343629P 20011024 (60)
US 2001-339266P 20011024 (60)
US 2001-349575P 20011029 (60)
US 2001-346357P 20011101 (60)
US 2002-373261P 20020417 (60)
DT Utility
FS APPLICATION
LREP MINTZ, LEVIN, COHN, FERRIS, GLOVSKY, AND POPEO, P.C., ONE FINANCIAL CENTER, BOSTON, MA, 02111
CLMN Number of Claims: 45
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 24097
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed herein are nucleic acid sequences that encode novel polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies that immunospecifically bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the novel polypeptide, polynucleotide, or antibody specific to the polypeptide. Vectors, host cells, antibodies and recombinant methods for producing the polypeptides and polynucleotides, as well as methods for using same are also included. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel ***human*** nucleic acids and proteins.

AB . . . further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel ***human*** nucleic acids and proteins.

SUMM . . . invention includes the use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a ***human*** disease, the disease being selected from a pathology associated with a polypeptide with an amino acid sequence selected from the. . .

SUMM . . . prevention is desired in an amount sufficient to treat or prevent the pathology in the subject. The subject could be ***human*** .

SUMM . . . provides an antibody that binds immunospecifically to a NOVX polypeptide. The NOVX antibody may be monoclonal, humanized, or a fully ***human*** antibody. Preferably, the antibody has a dissociation constant for the binding of the NOVX polypeptide to the antibody less than. . .

SUMM . . . provides for the use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a ***human*** disease, associated with a NOVX polypeptide. Preferably the therapeutic is a NOVX antibody.

SUMM . . . 8b CG142202-03 39 40 Cytokine Receptor

8c CG142202-02 41 42 CRL2 Precursor Cytokine Receptor

9a CG142621-01 43 44 CRL2 Precursor ***Human*** GTP binding protein

10a CG142761-01 45 46 Histocombatibility 13

11a CG143926-01 47 48 HLA-B7 alpha chain precursor

12a CG144193-01 49 50 Secreted phosphoprotein. . .

CG150637-02 149 150 Cytokine Receptor

40a CG150694-01 151 152 Cytokine Receptor

41a CG151069-01 153 154 Bone marrow secreted protein

42a CG151189-01 155 156 ***Human*** apoptosis protein (APOP-2)

43a CG151801-01 157 158 Laminin 5-Beta 3

44a CG165961-01 159 160 Fibulin 3

44b CG165961-02 161 162 Fibulin 3

44c CG165961-03. . . Receptor

45b CG171681-02 169 170 CRL2 Precursor Cytokine Receptor

45c CG171681-03 171 172 CRL2 Precursor Cytokine Receptor

46a CG173318-01 173 174 ***Human*** metabolism protein 16

47a CG51595-01 175 176 HLA-B7 alpha chain precursor

47b CG51595-03 177 178 HLA-B7 alpha chain precursor

47c CG51595-04 179 180. . .

SUMM . . . of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding ***human*** NOVX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in. . .

SUMM [0068] The nucleotide sequences determined from the cloning of the ***human*** NOVX genes allows for the generation of probes and primers designed for use in identifying and/or cloning NOVX homologues in. . .

SUMM [0069] Probes based on the ***human*** NOVX nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various. . .

SUMM [0073] In addition to the ***human*** NOVX nucleotide sequences of SEQ ID NO:2n-1, wherein n is an integer between 1 and 107, it will be appreciated. . . that lead to changes in the amino acid sequences of the NOVX polypeptides may exist within a population (e.g., the ***human*** population). Such genetic polymorphism in the NOVX genes may exist among individuals within a population due to natural allelic variation.. . .

SUMM . . . nucleic acid molecules encoding NOVX proteins from other species, and thus that have a nucleotide sequence that differs from a ***human*** SEQ ID NO:2n-1, wherein n is an integer between 1 and 107, are intended to be within the scope of. . . allelic variants and homologues of the NOVX cDNAs of the invention can be isolated based on their homology to the ***human*** NOVX nucleic acids disclosed herein using the ***human*** cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

SUMM [0076] Homologs (i.e., nucleic acids encoding NOVX proteins derived from species other than ***human***) or other related sequences (e.g., paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular ***human*** sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

SUMM . . . into a vector containing, e.g., a RNA pol III transcription unit from the smaller nuclear RNA (snRNA) U6 or the ***human*** RNase P RNA HI. One example of a vector system is the GeneSuppressorm RNA Interference kit (commercially available from Imgenex).. . .

SUMM . . . derived from a mammal, more preferably from a rodent such as a rat or mouse, and most preferably from a ***human*** . Where used for therapeutic treatment, the cells are preferentially autologous, although non-autologous cell sources are also contemplated as within the. . .

SUMM . . . Western blotting, ELISA, and the like. A subject sample of cells or tissues is taken from a mammal, preferably a ***human*** subject, suffering from a disease state. The NOVX ribopolynucleotide is used to produce siRNA constructs, that are specific for the. . .

SUMM . . . chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of ***human*** antibody species.

SUMM . . . of NOVX that is located on the surface of the protein, e.g., a hydrophilic region. A hydrophobicity analysis of the ***human*** NOVX protein sequence will indicate which regions of a NOVX polypeptide are particularly hydrophilic and, therefore, are likely to encode. . .

SUMM . . . protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of ***human*** origin are desired, or spleen cells or lymph node cells are used if non- ***human*** mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such. . . Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and ***human*** origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture. . .

SUMM . . . for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Manassas, Va. ***Human*** myeloma and mouse- ***human*** heteromyeloma cell lines also have been described for the production of ***human*** monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New. . .

SUMM . . . antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for ***human*** heavy and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison, Nature 368,. . .

SUMM [0181] The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or ***human*** antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the ***human*** against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab').sub.2 or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a ***human*** immunoglobulin, and contain minimal sequence derived from a non- ***human*** immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et. . . (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a ***human*** antibody. (See also U.S. Pat. No. 5,225,539.) In some instances, Fv framework residues of the ***human*** immunoglobulin are replaced by corresponding non- ***human*** residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR. . . and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non- ***human*** immunoglobulin and all or substantially all of the framework regions are those of a ***human*** immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fe), typically that of a ***human*** immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

SUMM [0182] ***Human*** Antibodies

SUMM [0183] Fully ***human*** antibodies essentially relate to antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from ***human*** genes. Such

antibodies are termed " ***human*** antibodies", or "fully ***human*** antibodies" herein. ***Human*** monoclonal antibodies can be prepared by the trioma technique; the ***human*** B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce ***human*** monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). ***Human*** monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using ***human*** hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming ***human*** B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R.. . .

SUMM [0184] In addition, ***human*** antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, ***human*** antibodies can be made by introducing ***human*** immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, ***human*** antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody. . .

SUMM [0185] ***Human*** antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully ***human*** antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding ***human*** heavy and light chain immunoglobulins are inserted into the host's genome. The ***human*** genes are incorporated, for example, using yeast artificial chromosomes containing the requisite ***human*** DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals. . . the Xenomouse.TM. as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully ***human*** immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example,. . . immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with ***human*** variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs. . .

SUMM [0187] A method for producing an antibody of interest, such as a ***human*** antibody, is disclosed in U.S. Pat. No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding. . .

SUMM [0192] Bispecific antibodies are monoclonal, preferably ***human*** or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the. . .

SUMM . . . the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal ***human*** T cells, as well as trigger the lytic activity of ***human*** cytotoxic lymphocytes against ***human*** breast tumor targets.

SUMM . . . to a, triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or ***Fc*** receptors for IgG (***Fc*** .gamma.R), such as ***Fc*** .gamma.RI (CD64), ***Fc*** .gamma.RII (CD32) and ***Fc*** .gamma.RIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be. . .

SUMM . . . to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the ***Fc*** region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability. . . as described in Wolff et al. Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual ***Fc*** regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230. . .

SUMM [0218] Antibodies of the invention, including polyclonal, monoclonal,

humanized and fully ***human*** antibodies, may used as therapeutic agents. Such agents will generally be employed to treat or prevent a disease or pathology. . .

SUMM . . . immunoassays are described, for example in "ELISA: Theory and Practice: Methods in Molecular Biology", Vol. 42, J. R. Crowther (Ed.) ***Human*** Press, Totowa, N.J., 1995; "Immunoassay", E. Diamandis and T. Christopoulus, Academic Press, Inc., San Diego, Calif., 1996; and "Practice and. . .

SUMM [0248] The host cells of the invention can also be used to produce non- ***human*** transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which NOVX protein-coding sequences have been introduced. Such host cells can then be used to create non- ***human*** transgenic animals in which exogenous NOVX sequences have been introduced into their genome or homologous recombinant animals in which endogenous. . . NOVX protein and for identifying and/or evaluating modulators of NOVX protein activity. As used herein, a "transgenic animal" is a non- ***human*** animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non- ***human*** primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of. . . one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non- ***human*** animal, preferably a mammal, more preferably a mouse, in which an endogenous NOVX gene has been altered by homologous recombination. . .

SUMM . . . fertilized oocyte (e.g., by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The ***human*** NOVX cDNA sequences, i.e., any one of SEQ ID NO:2n-1, wherein n is an integer between 1 and 107, can be introduced as a transgene into the genome of a non- ***human*** animal. Alternatively, a non- ***human*** homologue of the ***human*** NOVX gene, such as a mouse NOVX gene, can be isolated based on hybridization to the ***human*** NOVX cDNA (described further supra) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in. . .

SUMM . . . or substitution has been introduced to thereby alter, e.g., functionally disrupt, the NOVX gene. The NOVX gene can be a ***human*** gene (e.g., the cDNA of any one of SEQ ID NO:2n-1, wherein n is an integer between 1 and 107), but more preferably, is a non- ***human*** homologue of a ***human*** NOVX gene. For example, a mouse homologue of ***human*** NOVX gene of SEQ ID NO:2n-1, wherein n is an integer between 1 and 107, can be used to construct. . .

SUMM [0254] Clones of the non- ***human*** transgenic animals described herein can also be produced according to the methods described in Wilmut, et al., 1997. Nature 385:. . .

SUMM . . . examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% ***human*** serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and. . .

SUMM . . . detecting the induction of a reporter gene (comprising a NOVX-responsive regulatory element operatively linked to a nucleic acid encoding a ***detectable*** ***marker*** , e.g., luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

SUMM . . . thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual ***human*** chromosomes. Only those hybrids containing the ***human*** gene corresponding to the NOVX sequences will yield an amplified fragment.

SUMM [0295] Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., ***human*** and mouse cells). As hybrids of ***human*** and mouse cells grow and divide, they gradually lose ***human*** chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which ***human*** cells can, the one ***human*** chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single ***human*** chromosome or a small number of ***human*** chromosomes, and a full set of mouse chromosomes, allowing easy mapping

of individual genes to specific ***human*** chromosomes. See, e.g., D'Eustachio, et al., 1983. Science 220: 919-924. Somatic cell hybrids containing only fragments of ***human*** chromosomes can also be produced by using ***human*** chromosomes with translocations and deletions.

SUMM . . . to get good results at a reasonable amount of time. For a review of this technique, see, Verma, et al., ***HUMAN*** CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

SUMM . . . obtain such identification sequences from individuals and from tissue. The NOVX sequences of the invention uniquely represent portions of the ***human*** genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in. . .

SUMM . . . e.g., DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. See, e.g., Cronin, et al., 1996. ***Human*** Mutation 7: 244-255; Kozal, et al., 1996. Nat. Med. 2: 753-759. For example, genetic mutations in NOVX can be identified. . .

SUMM . . . model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in ***human*** subjects. Similarly, for in vivo testing, any of the animal model system known in the art may be used prior to administration to ***human*** subjects.

DETD . . .	946/947 associated protein #77--Mammalia, 947 aa. [WO200230268-A2, 18 APR. 2002]	0.0	1 . . . 947	947/947 (99%) (99%)	
AAB95517	***Human*** protein sequence SEQ ID NO: 18089--Homo sapiens, 875 aa. [EP1074617-A2, 7 FEB. 2001]	775 . . . 1606	59 . . . 854	399/835 (47%) 534/835 (63%)	0.0
AAO04442	***Human*** poly-peptide SEQ ID NO 18334--Homo sapiens, 112 aa. [WO200164835-A2, 7 SEP. 2001]	1190 . . . 1301	1 . . . 112	110/112 (98%) 110/112 (98%)	5e-56
ABG00933	Novel ***human*** diagnostic protein #924--Homo sapiens, 172 aa. [WO200175067-A2, 11 OCT. 2001]	109 . . . 258	2 . . . 145	101/150 (67%) 115/150 (76%)	9e-51
ABG07439	Novel ***human*** diagnostic protein #7430--Homo sapiens, 175 aa.. . .	1223 . . . 1348	4 . . . 131	61/128 (47%) 75/128	5e-24
DETD . . .	(Mouse), 1954 aa.		1 . . . 1954	(88%) 1818/1957 (92%)	
Q9H2Q8	GREB1a--Homo sapiens (***Human***), 1001 aa (fragment).		1 . . . 1001	999/1001 (99%) 999/1001 (99%)	0.0
O60321	KIAA0575 protein--Homo sapiens (***Human***), 947 aa.	1003 . . . 1949	1 . . . 947	946/947 (99%) 947/947 (99%)	0.0
Q9CYA3	8 days embryo cDNA, . . . clone: 5730583K22, full insert sequence--Mus musculus	1439 . . . 1949		471/511 (96%)	0.0

(Mouse), 511 aa.

Q9H2Q7	GREB1b--Homo sapiens (***Human***), 457 aa.	1 . . . 449	448/449 (99%)	0.0 (99%)
		1 . . . 449	448/449 (99%)	

DETD Identifier	Match [Patent #, Date]	Matched Residues	Expect Region	Value
AAB25582	ITGA11 protein encoded by ***human*** secreted protein gene #7--Homo sapiens, 1189 aa. [WO200029435-A1, 25 MAY 2000]	1 . . . 1189	1189/1189 (100%)	0.0 (100%)
		1 . . . 1189	1189/1189 (100%)	
ABG12949	Novel ***human*** diagnostic protein #12940--Homo sapiens, 1189 aa. [WO200175067-A2, 11 OCT. 2001]	1 . . . 1189	1188/1189 (99%)	0.0
		1 . . . 1189	1189/1189 (99%)	
AAU10551	***Human*** A259 polypeptide--Homo sapiens, 1188 aa. [WO200181414-A2, 1 NOV. 2001]	1 . . . 1189	1186/1189 (99%)	0.0
		1 . . . 1188	1187/1189 (99%)	
AAB50085	***Human*** A259-- Homo sapiens, 1188 aa. [WO200073339-A1, 7 DEC. 2000]	1 . . . 1189	1186/1189 (99%)	0.0
		1 . . . 1188	1187/1189 (99%)	
AAU14231	***Human*** novel protein #102-- Homo sapiens, 1188. . .	1 . . . 1189	1186/1189 (99%)	0.0
		1 . . . 1188	1187/1189 (99%)	

DETD Number	Match Organism/Length	Matched Residues	Expect Portion	Value
Q9UKX5	Integrin alpha-11 precursor--Homo sapiens (***Human***), 1189 aa.	1 . . . 1189	1189/1189 (100%)	0.0
		1 . . . 1189	1189/1189 (100%)	
CAD28200	Sequence 1 from Patent WO0181414-- Homo sapiens (***Human***), 1188 aa.	1 . . . 1189	1186/1189 (99%)	0.0
		1 . . . 1188	1187/1189 (99%)	
CAD28203	Sequence 19 from Patent WO0181414-- Mus musculus (Mouse), 1188 aa.	1 . . . 1189	1073/1189 (90%)	0.0
		1 . . . 1188	1130/1189 (94%)	
Q8WY18	MSTP018-- Homo sapiens (***Human***), 823 aa.	366 . . . 1189	822/824 (99%)	0.0
		1 . . . 823	823/824 (99%)	
O75578	Integrin alpha-10 precursor--Homo sapiens (***Human***), 1167 aa.	1 . . . 1170	513/1181 (43%)	0.0
		1 . . . 1150	723/1181 (60%)	

DETD			
	e-113		
	Willebrand/thrombosporin-like polypeptide - Homo sapiens, 235 aa. [WO200153485-A1, Jul. 26, 2001]	1 . . . 208	196/222 (87%)
AAM99920 (85%)	***Human*** polypeptide SEQ ID NO 36 - Homo sapiens, 272 aa. [WO200155173-A2,	384 . . . 592	185/217
	e-112		
		5 . . . 205	188/217 (86%)

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              Aug. 2, 2001]
AAM99933        ***Human***   polypeptide SEQ ID      384 . . . 592   181/217
       (83%)       e-110
              NO 49 - Homo sapiens, 212      5 . ..  .  .  . . . 190    178/204
       (86%)
              like mature protein sequence -
              Homo sapiens, 217 aa.
              [WO200153485-A1,
              Jul. 26, 2001]
ABG15393      Novel   ***human***   diagnostic         72 . . . 140    69/69
       (100%)      8e-39
              protein #15384 - Homo         959 . . . 1027   69/69 (100%)
. . .
DETD   . . . (91%)      0.0
           NT2RP7009498, weakly             1 . . . 589  558/607 (91%)
           similar to fibulin-1, isoform A
           precursor - Homo sapiens
           (  ***Human***  ), 955 aa.
Q9DBE2     1300015B04Rik protein - Mus       1 . . . 620  498/628 (79%)
       0.0
           musculus (Mouse), 608 aa.         1 ..  .  .  566 132/295 (43%)
           (Complement component 1, q
           subcomponent, receptor 1)
           (C1qRp) (C1qR(p))
           (C1q/MBL/SPA receptor)
           (CD93 antigen) (CDw93) -
           Homo sapiens (  ***Human***  ), 652
           aa.
Q9CXD8     6130401L20Rik protein - Mus       54 . . . 260  78/219 (35%)
       2e-29
           musculus (Mouse), 528 aa.         96 ..  .  .
DETD   . . . e-137
              protein 3 (EBI3) - Homo       1 . . . 229 229/229 (100%)
              sapiens, 229 aa.
              [WO9713859-A1,
              Apr. 17, 1997]
ABB81683        ***Human***   clone LO81-19a          1 . . . 229 228/229 (99%)
       e-136
              protein #1 - Homo sapiens,    1 . . . 229 229/229 (99%)
              229 aa. [WO200231114-A2,
              Apr. 18, 2002]
AAO14527        ***Human***   EBI-3 protein -         1 . . . 229 227/229 (99%)
       e-136
              Homo sapiens, 229 aa.         1 . . . 229 228/229 (99%)
              [WO200212282-A2,
              Feb. 14, 2001]
AAB36652        ***Human***   cytokine receptor       1 . . . 229 227/229 (99%)
       e-136
              subunit Eib3 protein SEQ ID   1 . . . 229.  .  .
DETD   . . . 4E


Public BLASTP Results for NOV4a

                                          NOV4a
Protein                                   Residues/     Identities/
Accession                                 Match         Similarities for the
       Expect
Number        Protein/Organism/Length     Residues      Matched Portion
       Value

Q75269          ***Human***   cytokine receptor        1 . . . 229  229/229
       (100%)       e-136
              (Epstein-Barr virus induced   1 . . . 229  229/229 (100%)
              gene 3) - Homo sapiens
              (  ***Human***  ), 229 aa.
Q14213        Cytokine receptor precursor-  1 . . . 229  227/229 (99%)
       e-135
              Homo sapiens (  ***Human***  ), 229      1 . . . 229  228/229
       (99%)
              aa.
O35228        Cytokine receptor-like        1 . . . 220  138/220 (62%)
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5e-75

molecule. . . Patent 1 . . . 67 67/67 (100%)

3e-34

WO0214358 - Homo sapiens 1 . . . 67 67/67 (100%)

(***Human***), 102 aa.

CAD44518 SI:bZ76A6.1 (novel protein 31 . . . 224 65/196 (33%)

5e-24

similar to vertebrate ciliary 5 . .. . .

DETD . . . Results for NOV5a

NOV5a

Residues/ Geneseq Identifier	Identities/ Protein/Organism/Length [Patent #, Date]	Match Residues	Similarities for the Matched Region	Expect Value
AAW37870	***Human*** protein comprising	1 . . . 1172	1161/1172 (99%)	0.0
	secretory signal amino acid sequence 7 - Homo sapiens, 1172 aa. [WO9811217-A2, Mar. 19, 1998]	1 . . . 1172	1161/1172 (99%)	
AAB48466	***Human*** laminin 5	4 . . . 1172	1151/1169 (98%)	0.0
	polypeptide, SEQ ID NO: 22 - Homo sapiens, 1174 aa. [WO200066731-A2, Nov. 9, 2000]	6 . . . 1174	1151/1169 (98%)	
AAB48462	***Human*** laminin 5	1 . . . 1172	1152/1172 (98%)	0.0
	polypeptide, SEQ ID NO: 14 - Homo sapiens, 1170 aa. [WO200066731-A2, Nov. 9, 2000]	1 . . . 1170	1155/1172 (98%)	
AAB48464	***Human*** laminin 5	4 . . . 1172	1152/1181 (97%)	0.0
	polypeptide, SEQ ID NO: 18 - Homo sapiens, 1186 aa. [WO200066731-A2, Nov. 9, 2000]	6 . . . 1186	1152/1181 (97%)	
AAB48465	***Human*** laminin 5	17 . . . 1172	1145/1156 (99%)	0.0
	polypeptide, SEQ ID NO: 20 -	12		
DETD . . .	(Laminin 5 beta 3) (Laminin B1k chain) (Kalinin B1 chain) - Homo sapiens (***Human***), 1172 aa.	1 . . . 1172	1161/1172 (99%)	
CAC17363	Sequence 21 from Patent	4 . . . 1172	1151/1169 (98%)	0.0
	WO0066731 precursor - Homo sapiens (***Human***), 1174 aa.	6 . . . 1174	1151/1169 (98%)	
CAC17359	Sequence 13 from Patent	1 . . . 1172	1152/1172 (98%)	0.0
	WO0066731 precursor - Homo sapiens (***Human***), 1170 aa.	1 . . . 1170	1155/1172 (98%)	
CAC17361	Sequence 17 from Patent	4 . . . 1172	1152/1181 (97%)	0.0
	WO0066731 precursor - Homo sapiens (***Human***), 1186 aa.	6 . . . 1186	1152/1181 (97%)	
CAC17362	Sequence 19 from Patent	17 . . . 1172	1145/1156 (99%)	0.0
	WO0066731 - Homo sapiens (***Human***), 1167 aa (fragment).	12 . . . 1167	1145/1156 (99%)	

DETD . . . Results for NOV6a

NOV6a

Residues/ Geneseq	Identities/ Protein/Organism/Length	Match	Similarities for the

Identifier	Expect Value	[Patent #, Date]	Residues	Matched Region
AAB48077	(100%)	***Human*** extracellular signaling molecule (EXCS) (ID 1359783CD1) - Homo sapiens, 493 aa. [WO200070049-A2, Nov. 23, 2000]	1 . . . 493	493/493 (100%)
AAB72892	(100%)	***Human*** EFEMP1 - Homo sapiens, 493 aa.	1 . . . 493	493/493 (100%)
AAY08066	(100%)	***Human*** EGF-like protein S1-5 fragment #1 encoded by GEN12205 cDNA - Homo sapiens, 350 aa. [WO9914241-A2, Mar. 25, 1999]	144 . . . 493 1 . . . 350	350/350 350/350 (100%)
AAY08490	(98%)	***Human*** EGF-like protein S1-5 fragment #2 encoded by	3 . . . 346 1 . . . 348. . .	344/348
DETD	. . .	protein 1 precursor (Fibulin-3) (FIBL-3) (Fibrillin-like protein) (Extracellular protein S1-5) - Homo sapiens (***Human***), 493 aa.	1 . . . 493	493/493 (100%)
O35568	0.0	EGF-containing fibulin-like extracellular matrix protein 1 precursor (Fibulin-3) (FIBL-3) (T16 protein) - Rattus norvegicus (Rat), 493 aa.	1 . . . 493 1 . . . 493	459/493 (93%) 476/493 (96%)
I38449	(100%)	extracellular protein - ***human*** , 387 aa.	107 . . . 493 1 . . . 387	387/387 387/387 (100%)
AAH31184		Hypothetical protein -. . .		
DETD	. . .	1 . . . 418 epithelium-derived neurotrophic factor (PEDNF) - Homo sapiens, 418 aa. [WO9324529-A, Dec. 9, 1993]	1 . . . 418	418/418 (100%)
AAE10306	(99%)	***Human*** pigment epithelium derived growth factor (PEDF) -	1 . . . 418 1 . . . 418. . .	416/418
DETD	. . .	pigment	1 . . . 418	416/418 (99%)
	0.0	epithelial-differentiating factor precursor - ***human*** , 418 aa.	1 . . . 418	416/418 (99%)
P36955	0.0	Pigment epithelium-derived factor precursor (PEDF) (EPC-1) - Homo sapiens (***Human***), 418 aa.	1 . . . 418 1 . . . 418	414/418 (99%) 416/418 (99%)
Q96CT1	0.0	Hypothetical 46.4 kDa protein - Homo sapiens (***Human***), 418 aa.	1 . . . 418 1 . . . 418	413/418 (98%) 415/418 (98%)
O70629	0.0	Pigment epithelium-derived factor. . .	1 . . . 415	357/415 (86%)
DETD	. . .	for NOV8a		

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV8a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU77482	***Human*** thymic stromal lymphopoietin receptor (TSLPR)-FLAG polypeptide - Homo sapiens, 379 aa. [WO200200724-A2, Jan. 3, 2002]	1 . . . 371	370/371 (99%) 371/371 (99%)	0.0
AAU77481	***Human*** TSLPR (thymic stromal lymphopoietin receptor) polypeptide - Homo sapiens, 371 aa. [WO200200724-A2, Jan. 3, 2002]	1 . . . 371	370/371 (99%) 371/371 (99%)	0.0
AAU77220	***Human*** thymic stromal lymphopoietin receptor(TSLPR)-FLAG protein sequence - Homo sapiens, 379 aa. [WO200200723-A2, Jan. 3, 2002]	1 . . . 371	370/371 (99%) 371/371 (99%)	0.0
AAU77219	***Human*** thymic stromal lymphopoietin receptor (TSLPR). . .	1 . . . 371	370/371 (99%) 371/371 (99%)	0.0
DETD . . .	(99%) 0.0 PRECUSOR (IL-XR) (Thymic stromal LYMPHOPOIETIN protein receptor TSLPR) - Homo sapiens (***Human***), 371 aa.	1 . . . 371	371/371 (99%)	
Q9H5R3	CDNA: FLJ23147 fis, clone LNG09295 - Homo sapiens (***Human***), 232 aa.	1 . . . 176 1 . . . 175	161/176 (91%) 166/176 (93%)	2e-93
Q8R4S8	Thymic stromal lymphopoietin receptor -	24 . . . 371 28 . . . 360.	123/359 (34%)	5e-48
DETD . . .	for NOV9a			

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV9a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABB07505	***Human*** GTP-binding protein (GTPB) (ID: 4879308CD1) - Homo sapiens, 257 aa. [WO200204510-A2, Jan. 17, 2002]	1 . . . 256 1 . . . 257	160/259 (61%) 198/259 (75%)	2e-86
ABG34065	***Human*** Pro peptide #36 - Homo sapiens, 257 aa. [WO200224888-A2, Mar. 28, 2002]	1 . . . 256 1 . . . 257	160/259 (61%) 198/259 (75%)	2e-86
AAM41786	***Human*** polypeptide SEQ ID NO 6717 - Homo sapiens, 260 aa. [WO200153312-A1, Jul. 26, 2001]	1 . . . 256 4 . . . 260	160/259 (61%) 198/259 (75%)	2e-86
AAM40000	***Human*** polypeptide SEQ ID	1 . . . 256	160/259	

(61%) 2e-86
NO 3145 - Homo sapiens, 257 aa. [WO200153312-A1, Jul. 26, 2001] 1 . . . 257 198/259 (75%)
AAG67008 ***Human*** Yip1p28 polypeptide - Homo sapiens, 257 aa. 1 . . . 256 2e-86
1 . . . 257 198/259 (75%)
. . .
DETD 257 198/259 (75%)
similar to YIP1 protein (Similar to hypothetical protein AF140225) (Hypothetical 28.0 kDa protein) - Homo sapiens (***Human***), 257 aa.
AAK67644 Golgi membrane protein 1 . . . 256 159/259 (61%)
1e-84
SB140 - Homo sapiens (***Human***), 257 aa. 1 . . . 257 197/259 (75%)
Q9H338 Hypothetical 28.0 kDa protein - 1 . . . 256 159/259 (61%)
2e-84
Homo sapiens (***Human***), 257 aa. 1 . . . 257 195/259 (74%)
DETD . . . for NOV10a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV10a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB88567	***Human*** hydrophobic domain containing protein clone HP03010 #31 - Homo sapiens, 377 aa. [WO200112660-A2, Feb. 22, 2001]	1 . . . 379 1 . . . 375	353/379 (93%) 359/379 (94%)	0.0
AAB10549	***Human*** aspartate protease ps1 3 protein - Homo sapiens, 377 aa. [WO200043505-A2, Jul. 27, 2000]	1 . . . 379 1 . . . 375	353/379 (93%) 359/379 (94%)	0.0
AAY27132	***Human*** glioblastoma-derived polypeptide (clone OA004FG) - Homo sapiens, 377 aa. [WO9933873-A1, Jul. 8, 1999]	1 . . . 379 1 . . . 375	353/379 (93%) 359/379 (94%)	0.0
AAM93670	***Human*** polypeptide, SEQ ID NO: 3554 - Homo sapiens, 377 aa. [EP1130094-A2, Sep. 5, 2001]	1 . . . 379 1 . . . 375	352/379 (92%) 359/379 (93%)	0.0
AAY27133	***Human*** glioblastoma-derived polypeptide (clone OA004LD) -. . .	1 . . . 379 1 . . . 375	351/379 (92%) 357/379 (93%)	0.0

DETD . . . Expect

Number	Protein/Organism/Length	Residues	Portion	Value
Q95H87	Similar to histocompatibility 13 - Homo sapiens (***Human***), 377 aa.	1 . . . 379 1 . . . 375	354/379 (93%) 360/379 (94%)	0.0
Q8TCT9	Signal peptide peptidase - Homo sapiens (***Human***), 377 aa.	1 . . . 379 1 . . . 375	353/379 (93%) 359/379 (94%)	0.0

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                aa.
BAC11519        CDNA FLJ90802 fis, clone          1 . . . 379   352/379 (92%)
       0.0
                Y79AA1000226 - Homo               1 . . . 375   359/379 (93%)
                sapiens (  ***Human***  ), 377 aa.
Q9D8V0          1200006009Rik protein -           1 . . . 349   335/349 (95%)
       0.0
                Mus musculus (Mouse), 378         1 . . .  .  .
DETD   .  .  .  encoded by              21 . . . 196   173/176 (98%)     e-100
                genomic DNA encoding             187 . . . 362   175/176 (99%)
                    ***human***   histocompatibility
                antigen HLA-B 27 - Homo
                sapiens, 362 aa.
                [EP226069-A, Jun. 24, 1987]
AAP70590        Sequence of the   ***human***              21 . . . 196   172/176
       (97%)     e-99
                histocompatibility antigen    162 . . . 337   174/176 (98%)
                HLA B27 -.  .  .  (95%)    4e-97
                antigen - Homo sapiens, 362   188 . . . 362   172/175 (97%)
                aa. [EP354580-A,
                Feb. 14, 1990]
AAU32882        Novel   ***human***   secreted           22 . . . 196   169/176
       (96%)    1e-95
                protein #3373 - Homo              191 . . . 366   170/176 (96%)
.  .  .
DETD   .  .  .  Portion            Value

Q31603          Lymphocyte          21 . . . 196 176/176 (100%)     e-101
                antigen--Homo      187 . . . 362 176/176 (100%)
                sapiens (  ***Human***  ),
                362 aa.
Q29854          HLA-B alpha         21 . . . 196 176/176 (100%)     e-101
                chain antigen      187 . . . 362 176/176 (100%)
                precursor--Homo
                sapiens (  ***Human***  ),
                362 aa.
Q29861          HLA-BPOT            21 . . . 196 176/176 (100%)     e-101
                (classI)--Homo     187 . . . 362 176/176 (100%)
                sapiens (  ***Human***  ),
                362 aa.
Q29681          MHC class I         21 . . . 196 176/176 (100%)     e-101
                antigen heavy      187 . . . 362 176/176 (100%)
                chain precursor--
                Homo sapiens
                (  ***Human***  ), 362 aa.
Q29638          MHC class I         21 . . . 196 176/176 (100%)     e-101
                antigen--Homo      187 . . . 362 176/176 (100%)
                sapiens (  ***Human***  ),
                362 aa.
DETD   .  .  .  for NOV12a
                                    NOV12a        Identities/
                Protein/            Residues/     Similarities for
Geneseq         Organism/Length     Match         the Matched      Expect
Identifier      [Patent #, Date]    Residues      Region           Value

AAR10321          ***Human***   BMP--        1 . . . 184  183/211 (86%)     e-100
                Homo sapiens,      1 . . . 211  184/211 (86%)
                211 aa.
                [EP409472-A,
                23 JAN. 1991]
AAR10320          ***Human***   BMP--        1 . . . 184  183/211 (86%)     e-100
                Homo sapiens,      1 . . . 211  184/211 (86%)
                211 aa.
.  .  .
DETD   .  .  .  1 . . . 184  183/211 (86%)    3e-99
                phosphoprotein     1 . . . 211  184/211 (86%)
                24 precursor
                (SPP-24)--Homo
                sapiens (  ***Human***  ),
                211 aa.
AAH27494        RIKEN cDNA        11 . . . 184  121/200 (60%)    4e-59
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0610038O04 5 . . . 203 143/200 (71%)

. . .

DETD . . . for NOV13a

Geneseq Identifier	Protein/ Organism/Length [Patent #, Date]	NOV13a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU68550	***Human*** cytokine encoded by cDNA 790CIP2D_11 #1--Homo sapiens, 239 aa. [WO200175093-A1, 11 OCT. 2001]	novel 1 . . . 239 1 . . . 239	235/239 (98%) 237/239 (98%)	e-137
AAY53032	***Human*** protein clone di393_2 protein sequence SEQ ID NO: 70--Homo sapiens, 171 aa. [WO9957132-A1, 11 NOV. 1999]	secreted 69 . . . 239 1 . . . 171	168/171 (98%) 170/171 (99%)	1e-96
AAG00463	***Human*** protein, SEQ ID NO: 4544-- Homo sapiens, 101 aa. [EP1033401-A2, 6 SEP. 2000]	secreted 69 . . . 169 1 . . . 101	100/101 (99%) 100/101 (99%)	5e-55
AAY12683	***Human*** secreted protein SEQ ID NO: 273--Homo sapiens, 101 aa. [WO9906549-A2, 11 FEB. 1999]	5' EST 69 . . . 169 1 . . . 101	100/101 (99%) 100/101 (99%)	5e-55
AAM87953	***Human*** haematopoietic antigen SEQ. . .	immune/ 151 . . . 239 1 . . . 89	85/89 (95%) 88/89 (98%)	4e-44

DETD . . . Value

Q9HCV6	DJ1153D9.4 (Novel protein)--Homo sapiens (***Human***), 138 aa (fragment).	102 . . . 239 1 . . . 138	120/138 (86%) 126/138 (90%)	3e-66
Q9D9T2	1700029J11Rik protein--Mus	72 . . . 238 5 . . . 169	101/168 (60%) 122/168 (72%)	2e-46
. . . aa.				
Q9HCV7	DJ1153D9.3 (novel protein)--Homo sapiens (***Human***), 94 aa.	69 . . . 154 1 . . . 86	84/86 (97%) 84/86 (97%)	4e-44
Q96C09	Similar to neuronal thread protein-- Homo sapiens (***Human***), 106 aa.	69 . . . 156 1 . . . 88	80/88 (90%) 82/88 (92%)	8e-42
Q8YR98	Hypothetical protein A113550--	9 . . . 61 21 . . . 71	18/53 (33%) 31/53 (57%)	2.6

. . .

DETD . . . for NOV14a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV14a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU74426	***Human*** protein sequence #4,	1 . . . 243	242/243	

(99%) e-141
related to isolation of genes 1 . . . 243 242/243 (99%)
within SLE-1B - Homo
sapiens, 243 aa.
[WO200188200-A2,
Nov. 22, 2001]
AAW35857 ***Human*** CD48 for use in T 27 . . . 220 194/194
(100%) 1e-113
lymphocyte veto molecule - 1 . .. . . of genes 1 . . .
240 163/247 (65%)
within SLE-1B - Mus
musculus, 240 aa.
[WO200188200-A2,
Nov. 22, 2001]
AAG00342 ***Human*** secreted protein, 1 . . . 111 109/111
(98%) 4e-58
SEQ ID NO: 4423 - Homo 1 . . . 111 109/111 (98%)
sapiens, 111 aa.
[EP1033401-A2,
Sep. 6, 2000]
ABG47129 ***Human*** peptide encoded by 33 . . . 128 96/96
(100%) 4e-50
genome-derived single exon 1 . . . 96 96/96. . .
DETD . . . precursor 1 . . . 243 243/243 (100%)
(BCM1 surface antigen)
(Leucocyte antigen
MEM-102) (TCT.1)
(Antigen CD48) - Homo
sapiens (***Human***), 243 aa.
AAH30224 Similar to B-lymphocyte 1 . . . 148 132/148 (89%)
1e-69
activation marker BLAST-1 1 . . . 148 134/148 (90%)
(BCM1 surface antigen)
(Leucocyte antigen
MEM-102) (TCT.1)
(Antigen CD48) - Homo
sapiens (***Human***), 169 aa.
P18181 MRC OX-45 surface antigen 1 . . . 243 129/247 (52%)
5e-60
precursor (BCM1 surface 1 . .. . .
DETD . . . for NOV15a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV15a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABB90191	***Human*** polypeptide SEQ ID	1 . . . 202	202/221	
(91%)	e-112			
	NO 2967 - Homo sapiens	66 . . . 286	202/221 (91%)	
	286 aa. [WO200190304-A2,			
	Nov. 29, 2001]			
AAB75379	***Human*** secreted protein #38 -	1 . . . 202	202/221	
(91%)	e-112			
	Homo sapiens, 221 aa.	1 . . . 221	202/221 (91%)	
	[WO200100806-A2,			
	Jan. 4, 2001]			
AAE03982	***Human*** gene 43 encoded	1 . . . 202	202/221	
(91%)	e-112			
	secreted protein fragment,	1 . . . 221	202/221 (91%)	
	SEQ ID NO:180 - Homo			
	sapiens, 221 aa.			
	[WO200077022-A1,			
	Dec. 21, 2000]			
AAB25793	***Human*** secreted protein SEQ	1 . . . 202	202/221	
(91%)	e-112			
	ID #105 - Homo sapiens, 221	1 . . . 221	202/221 (91%)	
	aa. [WO200037491-A2,			
	Jun. 29, 2000]			
AAB53433	***Human*** colon cancer antigen	1 . . . 102	202/221	

(91%) e-112
protein sequence SEQ ID 28 . . . 248. . .

DETD . . . Matched Expect Number	Protein/Organism/Length	Residues	Portion	Value
Q9BRV3	Stromal cell protein - Homo	1 . . . 202	202/221 (91%)	
e-112	sapiens (***Human***), 221 aa.	1 . . . 221	202/221	
(91%)				
Q9UHQ3	Stromal cell protein - Homo	1 . . . 202	201/221 (90%)	
e-112	sapiens (***Human***), 221 aa.	1 . . . 221	202/221	
(90%)				
Q95KW8	Uterine stromal cell protein -	1 . . . 202	197/221. . .	221
198/221 (89%)				
	221 aa.			
Q9UHQ2	Stromal cell protein isoform -	1 . . . 202	171/202 (84%)	
1e-90	Homo sapiens (***Human***), 179	1 . . . 179	175/202	
(85%)				
	aa.			
Q9CXK4	Recombination activating	1 . . . 202	161/221 (72%)	4e-85
	gene. . .			

DETD . . . for NOV16a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV16a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABP41913	***Human*** ovarian antigen	1 . . . 92	92/92	
(100%)	2e-47			
	HVVBT41, SEQ ID	76 . . . 167	92/92 (100%)	
	NO:3045 - Homo sapiens,			
	353 aa. [WO200200677-A1,			
	Jan. 3, 2002]			
AAU02499	***Human*** trinucleotide repeat	1 . . . 92	92/92	
(100%)	2e-47			
	protein (TRP) - Homo	1 . . . 92	92/92 (100%)	
	sapiens, 278 aa.			
	[WO200130798-A1,			
	May 3, 2001]			
AAU12239	***Human*** PRO4409	1 . . . 92	92/92	
(100%)	2e-47			
	polypeptide sequence - Homo	1 . . . 92	92/92 (100%)	
	sapiens, 278 aa.			
	[WO200140466-A2,			
	Jun. 7, 2001]			
AAW78312	Fragment of ***human*** secreted	1 . . . 92	82/92	
(89%)	3e-39			
	protein encoded by gene 67 -	1 . . . 91. . .		
DETD . . .	(Unknown) (Protein for	1 . . . 92	92/92 (100%)	
	MGC:4122) (Protein for			
	MGC: 1220) (DJ475N16.1)			
	(CTG4A) - Homo sapiens			
	(***Human***), 278 aa.			
O15412	CTG4a - Homo sapiens	1 . . . 92	92/92 (100%)	
4e-47				
	(***Human***), 143 aa.	1 . . . 92	92/92	
(100%)				
Q9DAU1	1600025D17Rik protein	1 . . . 92	78/92 (84%)	
1e-36				
	(Putative. . . Patent	19 . . . 76	24/58 (41%)	
6e-06				
	EP1067182 - Homo sapiens	8 . . . 65	35/58 (59%)	
	(***Human***), 248 aa.			
Q8WUN9	Hypothetical 29.4 kDa protein -	19 . . . 76	24/58 (41%)	
6e-06				
	Homo sapiens (***Human***), 257	19 . . . 76	35/58	
(59%)				

aa (fragment).
DETD . . . for NOV17a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV17a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB65248	***Human*** PRO1141 (UNQ579)	1 . . . 221	220/246	
(89%)	e-120			
	protein sequence SEQ ID NO:303 - Homo sapiens, 247 aa. [WO200073454-A1, Dec. 7, 2000]	1 . . . 246	221/246 (89%)	
AAB94784	***Human*** protein sequence	1 . . . 221	220/246	
(89%)	e-120			
	SEQ ID NO: 15888 - Homo sapiens, 247 aa. [EP1074617-A2, Feb. 7, 2001]	1 . . . 246	221/246 (89%)	
AAM93680	***Human*** polypeptide, SEQ ID	1 . . . 221	220/246	
(89%)	e-120			
	NO: 3574 - Homo sapiens, 247 aa. [EP1130094-A2, Sep. 5, 2001]	1 . . . 246	221/246 (89%)	
AAU29137	***Human*** PRO polypeptide	1 . . . 221	220/246	
(89%)	e-120			
	sequence #114 - Homo sapiens, 247 aa. [WO200168848-A2, Sep. 20, 2001]	1 . . . 246	221/246 (89%)	
AAY57881	***Human*** transmembrane	1 . . . 221	220/246	
(89%)	e-120			
	protein HTMPN-5 - Homo	1 . . . 246	221/246 (89%)	

. . .

DETD	 240	240/265 (90%)		e-131
	protein (CGI-78 protein) - Homo sapiens (***Human***), 265 aa.	1 . . . 265	240/265 (90%)	
Q9BVG0	Similar to CGI-78 protein -	1 . . . 240	239/265 (90%)	
e-131				
	Homo sapiens (***Human***), 265 aa.	1 . . . 265	240/265	
(90%)				
Q8R1T3	CGI-78 protein - Mus	1 . . . 240	238/265 (89%). . .	
1 . . . 265	239/265 (89%)			
Q969R6	CGI-78 protein - Homo	1 . . . 221	220/246 (89%)	
e-119				
	sapiens (***Human***), 247 aa.	1 . . . 246	221/246	
(89%)				
CAC39761	Sequence 159 from Patent	1 . . . 221	219/246 (89%)	
e-118				
	EP1067182 - Homo sapiens (***Human***), 247 aa.	1 . . . 246	220/246 (89%)	

DETD . . . Results for NOV18a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV18a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABP48034	***Human*** polypeptide SEQ ID	28 . . . 165	137/138	
(99%)	3e-84			
	NO 464 - Homo sapiens, 243 aa. [US2002042386-A1, 11-APR-2002]	106 . . . 243	138/138 (99%)	
ABP47873	***Human*** polypeptide SEQ ID	28 . . . 165	137/138	
(99%)	3e-84			
	NO 303 - Homo sapiens, 246	109 . . . 246	138/138 (99%)	

aa. [US2002042386-A1,
11-APR-2002]
AAU98014 ***Human*** dendritic cell 28 . . . 165 137/138
(99%) 3e-84
immunoreceptor AJ133532 - 100 . . . 237 138/138 (99%)
Homo sapiens, 237 aa.
[WO200232958-A2,
25-APR-2002]
ABB90277 ***Human*** polypeptide SEQ ID 28 . . . 165 137/138
(99%) 3e-84
NO 2653 - Homo sapiens, 100 . . . 237 138/138 (99%)
237 aa. [WO200190304-A2,
29-NOV-2001]
AAU19814 ***Human*** novel extracellular 28 . . . 165 137/138
(99%) 3e-84
matrix protein, Seq ID No 106 . . . 243. . .
DETD . . . short 1 . . . 165 163/204 (79%) 8e-93
form - Homo sapiens 1 . . . 204 165/204 (79%)
(***Human***), 204 aa.
Q9UMR7 Dendritic cell 28 . . . 165 137/138 (99%) 9e-84
immunoreceptor - Homo 100 . . . 237 138/138 (99%)
sapiens (***Human***), 237 aa.
Q9UI34 C-type lectin superfamily 6 - 28 . . . 165 137/138 (99%) 9e-84
Homo sapiens (***Human***), 237 100 . . . 237 138/138
(99%)
aa.
Q9NS33 HDCGC13P - Homo sapiens 28 . . . 165 136/138 (98%) 3e-83
(***Human***), 237 aa. 100 . . . 237 137/138
(98%)
Q8WXW9 ***Fc*** - ***epsilon*** ***receptor*** III - 28 .
. . 156 128/129 (99%) 5e-78
Homo sapiens (***Human***), 230 100 . . . 228 129/129
(99%)
aa.
DETD . . . Results for NOV19a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV19a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAM39930	***Human*** polypeptide SEQ ID	1 . . . 198	198/216	
(91%)	e-107			
	NO 3075 - Homo sapiens,	1 . . . 216	198/216 (91%)	
	216 aa. [WO200153312-A1,			
	26-JUL-2001]			
ABB84847	***Human*** PRO1864 protein	1 . . . 198	198/234	
(84%)	e-105			
	sequence SEQ ID NO:62 -	1 . . . 234	198/234 (84%)	
	Homo sapiens, 234 aa.			
	[WO200200690-A2,			
	03-JAN-2002]			
ABB95453	***Human*** angiogenesis related	1 . . . 198	198/234	
(84%)	e-105			
	protein PRO1864 SEQ ID	1 . . . 234	198/234 (84%)	
	NO: 62 - Homo sapiens, 234			
	aa. [WO200208284-A2,			
	31-JAN-2002]			
AAB87532	***Human*** PRO 1864 - Homo	1 . . . 198	198/234	
(84%)	e-105			
	sapiens, 234 aa.	1 . . . 234	198/234 (84%)	
	[WO200116318-A2,			
	08-MAR-2001]			
AAM41716	***Human*** polypeptide SEQ ID	1 . . . 198	198/234	
(84%)	e-105			
	NO 6647 - Homo sapiens,	5		
DETD . . .	for 1 . . . 234 198/234 (84%)			
	MGC: 14607) (Similar to			
	steroidogenic acute regulatory			
	protein related) - Homo			

sapiens (***Human***), 234 aa.

Q99J63	Similar to RIKEN cDNA 0610035N01 gene - Mus	1 . . . 198 1	186/235 (79%)	1e-96

DETD . . . NOV20a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV20a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABB97369	Novel ***human*** protein SEQ ID NO: 637 - Homo sapiens, 541 aa. [WO200222660-A2, Mar. 21, 2002]	291 . . . 792 1 . . . 505	499/507 (98%) 500/507 (98%)	(98%) 0.0
AAB94004	***Human*** protein sequence SEQ ID NO: 14117 - Homo sapiens, 807 aa. [EP1074617-A2, Feb. 7, 2001]	19 . . . 745 27 . . . 755	445/735 565/735 (76%)	(60%) 0.0
AAB42245	***Human*** ORFX ORF2009 polypeptide sequence SEQ ID NO:4018 - Homo sapiens, 480 aa. [WO200058473-A2, Oct. 5, 2000]	19 . . . 472 3 . . . 480	440/482 442/482 (91%)	(91%) 0.0
ABG63456	***Human*** albumin fusion protein #131 - Homo sapiens, 318 aa. [WO200177137-A1, Oct. 18, 2001]	493 . . . 810 1 . . . 318	316/318 318/318 (99%)	(99%) 0.0
AAG71250	***Human*** gene 8-encoded secreted protein HCEIE80,	493 . . . 810 1 . . . 318	316/318 318/318 (99%)	(99%) 0.0

. . .

DETD . . . the Matched

Number	Protein/Organism/Length	Residues	Portion	Expect Value
CAD38916	Hypothetical protein - Homo sapiens (***Human***), 519 aa (fragment).	167 . . . 681 1 . . . 518	510/520 (98%) 512/520 (98%)	0.0
AAH30245	KIAA0792 gene product - Homo sapiens (***Human***), 807 aa.	19 . . . 745 27 . . . 755	449/735 (61%) 570/735 (77%)	0.0
O94886	KIAA0792 protein - Homo sapiens (***Human***), 807 aa.	19 . . . 745 27 . . . 755	448/735 (60%) 569/735 (76%)	0.0
Q91YT8	Hypothetical 91.9 kDa	19 . . . 745	446/735 (60%)	0.0
. . . clone		1 . . . 447	440/475 (92%)	0.0
	THYMU2005001 - Homo sapiens (***Human***), 491 aa.	1 . . . 471	441/475 (92%)	

DETD . . . for NOV21a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV21a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABG04763	Novel ***human*** diagnostic protein #4754 - Homo	432 . . . 2819 609 . . . 3046	1382/2497 (55%) 1762/2497 (70%)	0.0

```
            sapiens, 3048 aa.
            [WO200175067-A2,
            Oct. 11, 2001]
ABB97274    Novel   ***human***   protein SEQ       1591 . . . 2821 1231/1254
      (98%)      0.0
            ID NO: 549 - Homo sapiens       1 . . . 1254  1231/1254 (98%)
            1254 aa.
            [WO200222660-A2,
            Mar. 21, 2002]
ABG04764    Novel   ***human***   diagnostic         567 . . . 2276  893/1794
      (49%)      0.0
            protein #4755 - Homo           293 . . . 1962 1155/1794 (63%)
. . .
DETD  . . . kDa          432 . . . 2819 1385/2493 (55%)      0.0
          protein - Homo sapiens          577 . . . 3010 1764/2493 (70%)
          (  ***Human***  ), 3012 aa.
O94915    KIAA0826 protein - Homo        1615 . . . 2821 1207/1236 (97%)
      0.0
          sapiens (  ***Human***  ), 1236 aa         1 . . . 1236  1207/1236
      (97%)
          (fragment).
O14572    WUGSC:H_248015.1                449 . . . 2276 1090/1892 (57%)
      0.0
          protein - Homo sapiens           1 . . . 1849  1375/1892 (72%)
          (  ***Human***  ), 1849 aa
          (fragment).
Q91ZH1    DM505L19.1 (Novel              1226 . . . 2819  877/1652 (53%)
      0.0
          protein) - Mus musculus          1 . .. . . kDa          1591 . .
      . 2385  795/795 (100%)      0.0
          protein - Homo sapiens           1 . . . 795    795/795 (100%)
          (  ***Human***  ), 795 aa (fragment).
DETD  . . . for NOV22a
```

Geneseq Identifier	Protein/ Organism/Length [Patent #, Date]	NOV22a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU12196	***Human*** polypeptide sequence--Homo sapiens, 477 aa.. ID NO 984--Drosophila melanogaster, 3060 aa. [WO200171042-A2, 27 SEP. 2001]	PRO4799 899 . . . 1280 94 . . . 477 SEQ 18 . . . 536	375/384 (97%) 377/384 282/529 (52%)	0.0
AAU72890	***Human*** partial protein sequence #2--Homo sapiens, 1103 aa. [WO200183782-A2, 8 NOV. 2001]	metallo- 29 . . . 549 550 . . . 1064	201/529 (37%) 272/529 (50%)	e-105
AAB74945	***Human*** type metal protease MDTS2 protein. . .	ADAM 29 . . . 549 550 . . . 1064	201/529 (37%) 272/529	e-105

DETD Number	Match Organism/Length	Matched Residues	Expect Portion	Value
O95428	Hypothetical 133.5 kDa protein--Homo sapiens (***Human***), 1235 aa.	1 . . . 1280 1 . . . 1235	1136/1321 (85%) 1146/1321 (85%)	0.0
Q9EPX2	Papilin--Mus musculus (Mouse),	5 . . . 1280	952/1283 (74%)	0.0

. . .
DETD . . . (87%) 0.0
ORF protein-- 14 . . . 404 344/391 (87%)
Mus sp, 404 aa.
[WO200214366-
A2, 21 FEB.
2002]
AAG79086 ***Human*** DC- 1 . . . 345 344/391 (87%) 0.0
SIGN, a dendritic 14 . . . 404 344/391 (87%)
cell-speciific
C-type lectin--
Homo sapiens,
404 aa.
[WO200164752-
A2, 7 SEP.
2001]
AAB28614 ***Human*** C-type 1 . . . 345 344/391 (87%) 0.0
lectin receptor-- 14 . . . 404 344/391 (87%)
Homo sapiens,
. . .
DETD . . . 14 . . . 404 344/391 (87%)
type-C (Probable
mannose-binding
C-type lectin DC-
SIGN) (MDC-
SIGN1A type I
isoform)--Homo
sapiens (***Human***),
404 aa.
Q96QQ1 MDC-SIGN1B type 3 . . . 345 342/389 (87%) 0.0
I isoform--Homo 16 . . . 404 342/389 (87%)
sapiens (***Human***),
404 aa.
Q96QQ8 MDC-SIGN1A type 1 . . . 345 338/391 (86%) 0.0
II isoform--Homo 14 . . . 398 338/391 (86%)
sapiens (***Human***),
398 aa.
Q95LC6 Dendritic cell- 1 . . . 345 319/368 (86%) 0.0
specific ICAM-3 14 . . . 381 332/368. . .
DETD 422 284/313 (90%)
encoded by DNA
clone
P0248_B04--
Rattus
norvegicus,
422 aa.
[WO200174901-
A2, 11 OCT.
2001]
AAM93823 ***Human*** poly- 1 . . . 308 158/340 (46%) 1e-76
peptide, SEQ ID 110 . . . 446 206/340 (60%)
NO: 3881--
Homo sapiens,
450 aa.
[EP1130094-A2,
5 SEP. 2001]
ABG65106 ***Human*** albumin 1 . . . 308 157/340 (46%) 4e-76
fusion protein 94 . . . 430 205/340 (60%)
#1781--Homo
sapiens, 434 aa.
[WO200177137-
A1, 18 OCT.
2001]
AAE07112 ***Human*** gene 6 1 . . . 308 157/340 (46%) 4e-76
encoded secreted 131 . . . 467 205/340 (60%)
protein fragment,
SEQ ID NO:
129--Homo
sapiens, 471 aa.
[WO200154708-
A1, 2 AUG.

	2001]			
AAE07056	***Human*** gene 6 encoded secreted	1 . . . 308 94 . . . 430	157/340 (46%) 205/340 (60%)	4e-76
. . .				
DETD	. . . 1 . . . 313	311/313	0.0	
	fis, clone SKMUS2000679-- Homo sapiens (***Human***), 313 aa.	1 . . . 313	(99%) 313/313 (99%)	
BAC05248	CDNA FLJ40846 fis, clone TRACH2014544-- Homo sapiens (***Human***), 422 aa.	1 . . . 313 110 . . . 422	273/313 (87%) 284/313 (90%)	e-161
Q8WVJ5	Similar to RIKEN cDNA 2310035L15 gene-- Homo sapiens (***Human***), 200 aa.	114 . . . 313 1 . . . 200	200/200 (100%) 200/200 (100%)	e-111
AAH22603	Hypothetical protein--Mus musculus (Mouse),	114 . . . 311 1 . . . 198.	177/198 (89%) . .	1e-97
DETD	. . . for NOV25a			

Geneseq Identifier	Protein/ Organism/Length [Patent #, Date]	NOV25a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABG65069	***Human*** albumin fusion protein #1744--Homo sapiens, 280 aa. [WO200177137-A1, 18 OCT. 2001]	21 . . . 119 182 . . . 280	95/99 (95%) 97/99 (97%)	8e-55
ABB89811	***Human*** poly-peptide SEQ ID NO 2187--Homo sapiens, 173 aa. [WO200190304-A2, 29 NOV. 2001]	21 . . . 119 75 . . . 173	95/99 (95%) 97/99 (97%)	8e-55
ABB97380	Novel ***human*** protein SEQ ID NO: 648--Homo sapiens, 493 aa. [WO200222660-A2, 21 MAR. 2002]	21 . . . 119 395 . . . 493	95/99 (95%) 97/99 (97%)	8e-55
AAE07114	***Human*** gene 9 encoded secreted protein fragment, SEQ ID NO: 131--Homo sapiens, 311 aa. [WO200154708-A1, 2 AUG. 2001]	21 . . . 119 213 . . . 311	95/99 (95%) 97/99 (97%)	8e-55
AAE07059	***Human*** gene 9 encoded secreted protein	21 . . . 119 182 . . . 280	95/99 (95%) 97/99 (97%)	8e-55
. . .				
DETD	. . . protein (Translation of cDNA KAT07271 (Em: AK000484))) (Hypothetical 55.7 kDa protein)-- Homo sapiens (***Human***), 493 aa.	395 . . . 493	97/99 (97%)	
Q9NX26	CDNA FLJ20477	21 . . . 119	95/99 (95%)	2e-54

fis, clone KAT07271--Homo sapiens (***Human***), 493 aa. 395 . . . 493 97/99 (97%)

Q9U3X2 VEGETABLE precursor-- Drosophila 9 . . . 119 38/123 (30%) 0.057
361 . . . 449 50/123 (39%)

. . .

DETD . . . NOV26a

Geneseq Identifier	Protein/ Organism/Length [Patent #, Date]	NOV26a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAW13131	Partial ***human*** cadherin-6--Homo sapiens, 414 aa. [US5597725-A, 28 JAN. 1997]	79 . . . 334 159 . . . 414	256/256 (100%) 256/256 (100%)	e-148
AAW25659	***Human*** cadherin-6--Homo sapiens, 414 aa. [US5646250-A, 8 JUL. 1997]	79 . . . 334 159 . . . 414	256/256 (100%) 256/256 (100%)	e-148
AAR43564	***Human*** cadherin-6--Homo sapiens, 391 aa. [WO9321302-A, 28 OCT. 1993]	79 . . . 311 159 . . . 391	233/233 (100%) 233/233 (100%)	e-133
ABP47864	***Human*** polypeptide SEQ ID NO 294--Homo sapiens, 358 aa. [US2002042386-A1, 11 APR. 2002]	77 . . . 334 101 . . . 358	212/258 (82%) 238/258 (92%)	e-125
AAU19644	***Human*** novel extra-cellular matrix protein, Seq ID No	77 . . . 334 101	212/258 (82%)	e-125

DETD . . . 79 . . . 334 256/256 (100%) e-147
precursor (Kidney-cadherin) (K-cadherin)-- Homo sapiens (***Human***), 790 aa. 535 . . . 790 256/256 (100%)

P97326 Cadherin-6 precursor (Kidney-cadherin) 79 . . . 334 246/256 (96%) e-143
535 . . . 790 253/256 (98%)

. . .

DETD . . . Results for NOV27a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV27a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB65224	***Human*** PRO1138 (UNQ576) protein sequence SEQ ID NO:253 - Homo sapiens, 335 aa. [WO200073454-A1, Dec. 7, 2000]	1 . . . 335 1 . . . 335	335/335 (100%) 335/335 (100%)	0.0
AAB87548	***Human*** PRO1138 - Homo sapiens, 335 aa. [WO200146260-A2. Jun. 28, 2001]	1 . . . 335 1 . . . 335 1 . . . 335	335/335 (100%) 335/335. . . . . . 335/335 (100%)	0.0 0.0
AAU29119	***Human*** PRO polypeptide	1 . . . 335	335/335	

(100%) 0.0
sequence #96 - Homo 1 . . . 335 335/335. . .
DETD 335 335/335 (100%)
protein) (NK cell receptor)
(Membrane protein
FOAP-12) (CD2-like
receptor activating cytotoxic
cells) - Homo sapiens
(***Human***), 335 aa.
Q9NY08 19A protein - Homo sapiens 1 . . . 335 334/335 (99%)
0.0
(***Human***), 335 aa. 1 . . . 335 335/335
(99%)
Q9NY23 19A24 protein - Homo 1 . . . 316 273/316 (86%)
e-152
sapiens (***Human***), 328 aa. 1 . . . 281 276/316
(86%)
AAH27867 19A24 protein - Homo 1 . . . 257 257/257 (100%)
e-149
sapiens (***Human***), 296 aa. 1 . . . 257 257/257
(100%)
CAD39085 Hypothetical protein - Homo 120 . . . 335 212/217 (97%)
e-123
sapiens (***Human***), 228 aa. 12 . . . 228 214/217
(97%)
DETD . . . Results for NOV28a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV28a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU74822 (26%)	***Human*** REPTR 5 protein - Homo sapiens, 173 aa.	6 . . . 150 3	42/158 2e-07	
DETD	. . . 1 . . . 167 166/167 (99%)		2e-93	
	1700071E18 gene - Homo sapiens (***Human***), 167 aa.	1 . . . 167	167/167 (99%)	
Q9D9H2	1700071E18Rik protein - Mus musculus (Mouse), 167	1 . . . 167	117/167 (70%)	2e-65

DETD . . . Results for NOV29a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV29a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB65220 (97%)	***Human*** PRO1383 (UNQ719) protein sequence SEQ ID NO:241 - Homo sapiens, 423 aa. [WO200073454-A1, Dec. 7, 2000]	1 . . . 435 1 . . . 423	423/435 423/435 (97%)	0.0
AAM25558 (97%)	***Human*** protein sequence SEQ ID NO: 1073 - Homo sapiens, 468 aa. [WO200153455-A2, Jul. 26, 2001]	1 . . . 435 46 . . . 468	423/435 423/435 (97%)	0.0
AAU29113 (97%)	***Human*** PRO polypeptide sequence #90 - Homo	1 . . . 435 1 . . . 423	423/435 423/435. . .	0.0
423/435 (97%)	PRO1383 - Homo sapiens, 423 aa. [WO9963088-A2, Dec. 9, 1999]	1 . . . 423	423/435 (97%)	0.0
ABG43580 (100%)	***Human*** peptide encoded by	185 . . . 239	55/55	7e-24

genome-derived single exon 1 . . . 55 55/55. . .
DETD . . . the Expect
Number Protein/Organism/Length Residues Matched Portion
Value

CAD39014 Hypothetical protein - Homo 1 . . . 435 435/435 (100%)
0.0
sapiens (***Human***), 435 aa. 1 . . . 435 435/435
(100%)
AAH30793 Similar to QNR-71 protein - 1 . . . 435 423/435 (97%)
0.0
Homo sapiens (***Human***), 423 1 . . . 423 423/435
(97%)
aa.
CAD38628 Hypothetical protein - Homo 27 . . . 435 396/409 (96%)
0.0
sapiens (***Human***), 397 aa 1 . . . 397 396/409
(96%)
(fragment).
AAM31285 Surface layer protein B - 177. . . 339 40/166. . . 150 .
. . 212 23/64 (35%) 0.001
(transmembrane) nmb - 254 . . . 317 36/64 (55%)
Homo sapiens (***Human***), 572
aa.
DETD . . . Results for NOV30a

NOV30a
Identities/
Residues/
Similarities for
Geneseq Protein/Organism/Length Match the
Matched Expect
Identifier [Patent #, Date] Residues Region
Value

AAG76160 ***Human*** colon cancer antigen 175 . . . 206
29/32 (90%) 5e-08
protein SEQ ID NO:6924 - 12 . . .. . .
DETD . . . the Matched Expect
Number Protein/Organism/Length Residues Portion
Value

Q14159 KIAA0146 protein - Homo 1 . . . 851 850/851 (99%)
0.0
sapiens (***Human***), 918 aa 4 . . . 854 851/851
(99%)
(fragment).
Q8R305 Hypothetical 43.0 kDa protein - 527 . . . 851. . . . 325
258/325 (78%)
aa.
Q96BI5 Hypothetical 23.1 kDa protein - 701 . . . 851 150/151 (99%)
1e-82
Homo sapiens (***Human***), 218 4 . . . 154 151/151
(99%)
aa (fragment).
P97399 Dentin sialophosphoprotein 86 . . . 196 29/112 (25%)
0.029
. . . 32/136 (23%) 0.051
(MYT1) (MYTI) (Proteolipid 221 . . . 355 65/136 (47%)
protein binding protein)
(PLPB1) - Homo sapiens
(***Human***), 1121 aa.
DETD . . . Results for NOV31a

NOV31a Identities/
Residues/ Similarities for
Geneseq Protein/Organism/Length Match the Matched
Expect
Identifier [Patent #, Date] Residues Region
Value

ABP43105 ***Human*** ovarian antigen 1 . . . 130 104/130
(80%) 3e-56

HVCBB19, SEQ ID NO:4237 - Homo sapiens, 143 aa. [WO200200677-A1, Jan. 3, 2002] 11 . . . 140 113/130 (86%)

AAE13797 ***Human*** lung tumour-specific protein SALT-T8 - Homo sapiens, 133 aa. [WO200172295-A2, Oct. 4, 2001] 1 . . . 130 104/130 (80%) 3e-56

1 . . . 130 113/130 (86%)

AAB44456 ***Human*** lung tumour-specific antigen encoded by cDNA #71 - Homo sapiens, 133 aa. [WO200060077-A2, Oct. 12, 2000] 1 . . . 130 104/130 (80%) 3e-56

1 . . . 130 113/130 (86%)

AAY29544 ***Human*** lung tumour protein SALT-T8 predicted amino 1 . . . 130 104/130 (80%) 3e-56

1 . . . 130 113/130. . . .

DETD . . . transmembrane protein 3 (Interferon-inducible protein 1-8U) - Homo sapiens (***Human***), 133 aa. 1 . . . 130 113/130 (86%)

AAH22439 Interferon induced transmembrane protein 3 (1-8U) - Homo sapiens (***Human***), 133 aa. 1 . . . 130 103/130 (79%) 6e-55

1 . . . 130 112/130 (85%)

S17182 interferon-induced protein 1-8U - ***human*** , 133 aa. 1 . . . 130 102/130 (78%) 8e-55

1 . . . 130 112/130 (85%)

Q01629 Interferon-induced transmembrane protein 2 (Interferon-inducible protein 1-8D) - Homo sapiens (***Human***), 132 aa. 1 . . . 133 98/133 (73%) 2e-51

1 . . . 132 110/133 (82%)

Q95MQ3 Interferon-induced protein 1-8U - Bos taurus (Bovine), 124 aa. 1 . . . 124 78/124 (62%) 9e-39

1 . . . . . .

DETD . . . Results for NOV32a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV32a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU12071	***Human*** PHT1 variant protein from Caco-2 cells - Homo sapiens, 577 aa. [WO200192468-A2, Dec. 6, 2001]	19 . . . 138 14 . . . 142	40/134 (29%) 54/134 (39%)	3.3
AAU12070	***Human*** PHT1 variant protein from BeWo cells - Homo sapiens, 577 aa. [WO200192468-A2, Dec. 6, 2001]	19 . . . 138 14 . . . 142	40/134 (29%) 54/134 (39%)	3.3
AAU12069	***Human*** PHT1 protein splice variant - Homo sapiens, 295 aa. [WO200192468-A2, Dec. 6, 2001]	19 . . . 138 14 . . . 142	40/134 (29%) 54/134 (39%)	3.3
AAU12068	***Human*** PHT1 protein isolated from Caco-2 cells - Homo	19 . . . 138 14	40/134 (29%)	3.3

DETD . . . 33D

Geneseq Results for NOV33a

NOV33a Identities/
Geneseq Protein/Organism/length Residues/Match Similarities for
the Expect
Identifier [Patent #, Date] Residues Matched Region
Value

AAY44897 ***Human*** PB39 protein 1 . . . 514
514/559 (91%) 0.0
dysregulated in prostate 1 . . . 559 514/559 (91%)
cancer - Homo sapiens, 559
aa. [W0200005376-A1,
03 Feb. 2000]
AAW64554 ***Human*** liver cell clone 1 . . . 514
514/559 (91%) 0.0
HP10301 protein - Homo 1 . . . 559 514/559 (91%)
sapiens, 559 aa.
[W09821328-A2,
22 May 1998]
AAY44898 ***Human*** PB39 variant protein 1 . . . 467
467/512 (91%) 0.0
dysregulated in prostate 1 . . . 512 467/512 (91%)
cancer - Homo sapiens, 560
aa. [W0200005376-A1,
03 Feb. 2000]
AAB94537 ***Human*** protein sequence 68 . . . 514
447/447 (100%) 0.0
SEQ ID NO: 15277 - Homo 39 . . . 485 447/447 (100%)
sapiens, 485 aa.
[EP1074617-A2,
07 Feb. 2001]
AAE05505 Mature ***human*** HC-like 68 . . . 495
250/436 (57%) e-38
protein #2 - Homo sapiens, 85 . . . 505 320/436. . .
DETD 514 514/559 (91%) 0.0
OVEREXPRESSED gene 1) 1 . . . 559 514/559 (91%)
- Homo sapiens (***Human***),
559 aa.
Q9D0H7 2610016F07Rik protein - 11 . . . 512 417/552 (75%)
0.0
Mus musculus (Mouse), 654 101 . . . . . kDa 1 . .
. 495 297/560 (53%) e-154
protein - Homo sapiens 1 . . . 545 374/560 (66%)
(***Human***), 569 aa.
BAC11450 CDNA FLJ90692 fis, clone 68 . . . 495 250/436 (57%)
e-138
PLACE1006443, weakly 16 . . . 436 320/436 (73%)
similar to Homo sapiens
PB39 mRNA - Homo sapiens
(***Human***), 460 aa.
BAC11383 CDNA FLJ90587 fis, clone 68 . . . 495 249/436 (57%)
e-137
PLACE1000914, weakly 16 . . . 436 320/436 (73%)
similar to Homo sapiens
PB39 mRNA - Homo sapiens
(***Human***), 460 aa.
DETD . . . for NOV34a

NOV34a
Residues/ Identities/
Geneseq Protein/Organism/Length Match Similarities for the
Expect
Identifier [Patent #, Date] Residues Matched Region
Value

AAO15149 ***Human*** cystatin D protein 1 . . . 142 142/142
(100%) 2e-80
sequence - Homo sapiens, 1 . . . 142 142/142 (100%)
142 aa. [US2002052476-A1,
02 May 2002]

AAE02408 ***Human*** cystatin D precursor 1 . . . 142 142/142 (100%) 2e-80
protein - Homo sapiens, 142 aa. [US6235708-B1, 22 May 2001] 1 . . . 142 142/142 (100%)
AAE04437 ***Human*** cystatin D 1 . . . 142 142/142 (100%) 2e-80
homologue protein - Homo sapiens, 142 aa. [US6245529-B1, 12 Jun. 2001] 1 . . . 142 142/142 (100%)
AAE11210 ***Human*** cystatin D (CysD) 1 . . . 142 142/142 (100%) 2e-80
protein - Homo sapiens, 142 aa. [US6300477-B1, 09 Oct. 2001] 1 . . . 142 142/142 (100%)
AAY81137 ***Human*** wild-type cystatin D 21 . . . 142 122/122 (100%) 3e-68
- Homo sapiens, 122 aa. 1 . . . . . .

DETD . . . Results for NOV34a

Protein Accession Number	Protein/Organism/Length	NOV34a Residues/Match Residues	Identities/ Similarities for the Matched Portion	Expect Value
A47142	cystatin D precursor - ***human*** , 142 aa.	1 . . . 142	142/142 (100%)	7e-80
P28325	Cystatin D precursor - Homo sapiens (***Human***), 142 aa.	1 . . . 142 1 . . . 142	141/142 (99%) 141/142 (99%)	6e-79
P09228	Cystatin SA precursor (Cystatin S5) - Homo sapiens (***Human***), 141 aa.	1 . . . 141 1 . . . 141	80/141 (56%) 108/141 (75%)	2e-42
P01036	Cystatin S precursor (Salivary acidic protein-1) (Cystatin SA-III) - Homo sapiens (***Human***), 141 aa.	1 . . . 141 1 . . . 140	79/141 (56%) 109/141 (77%)	2e-41
P01037	Cystatin SN precursor (Salivary cystatin SA-1) (Cystain SA-I) - Homo sapiens (***Human***), 141 aa.	5 . . . 141 5 . . . 140	78/137 (56%) 105/137 (75%)	5e-40

DETD . . . 35C

Geneseq Results for NOV35a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV35a Residues/Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAW62612	***Human*** glutamate-binding protein (HGLUBP) - Homo sapiens, 369 aa. [W09821241-A1, 22 May 1998]	1 . . . 341 1 . . . 365	337/365 (92%) 337/365 (92%)	0.0
ABB12050	***Human*** leukocyte HP00804 protein homologue, SEQ ID NO:2420 - Homo sapiens, 461 aa. [WO200157188-A2, 09 Aug. 2001]	49 . . . 496 1 . . . 461	339/463 (73%) 350/463 (75%)	e-180
AAW64535	***Human*** leukocyte cell clone	2 . . . 341	293/364 (80%)	e-161

HP00804 protein - Homo 4 . . . 367 297/364 (81%)
sapiens, 371 aa.
[WO9821328-A2,
22 May 1998]
AAY48255 ***Human*** prostate 50 . . . 328
240/304 (78%) e-129
cancer-associated protein 41 1 . . . 304 246/304 (79%)
-. . .
DETD . . . receptor 197 . . . 399 172/207 (83%) 4e-83
glutamate-binding 6 . . . 208 178/207 (85%)
chain--Homo
sapiens (***Human***),
208 aa
(fragment).
AAM68613 CG3798-PA-- 67 . . . 344 134/291 (46%) 3e-65
Drosophila 25 . . . 312 190/291. . .
DETD . . . 416 298/370 (80%) 0.0
sequence--Homo 271 . . . 633 308/370 (82%)
sapiens, 884 aa.
[WO200181578-
A2, 1 NOV.
2001]
AAB60394 ***Human*** nurse cell 64 . . . 416 298/370 (80%) 0.0
receptor 202 . . . 564 308/370 (82%)
B6TNC#10a,
SEQ ID NO:
24--Homo
sapiens, 866 aa.
[JP2000308492-
A, 7 NOV. 2000]
AAB60393 ***Human*** nurse cell 64 . . . 416 298/370 (80%) 0.0
receptor 202 . . . 564 308/370 (82%)
B6TNC#10,
SEQ ID NO:
21--Homo
sapiens, 866 aa.
[JP2000308492-
A, 7 NOV. 2000]
AAB60395 ***Human*** nurse cell 64 . . . 416 298/375 (79%) 0.0
receptor 202 . . . 569 308/375 (81%)
B6TNC#10b,
SEQ. . .
DETD . . . 113 . . . 416 282/304 (92%) 0.0
(Protein for 5 . . . 296 286/304 (93%)
IMAGE:
4125591)--Homo
sapiens (***Human***),
598 aa
(fragment).
CAD29035 Sequence 17 1 . . . 205 153/205 (74%) 2e-88
from Patent 1 . . . 175 158/205 (76%)
WO0214358--
Homo sapiens
(***Human***), 254 aa.
BAC02696 SREC-5--Homo 28 . . . 414 172/462 (37%) 7e-76
sapiens (***Human***), 6 . . . 455 224/462 (48%)
744 aa.
Q14162 Endothelial cells 35 . . . 414 153/405 (37%) 2e-75
scavenger 154 . . . 541 195/405 (47%)
receptor precursor
(Acetyl LDL
receptor)--Homo
sapiens (***Human***),
830 aa.
BAC02694 SREC-3--Homo 35 . . . 367 130/355 (36%) 1e-64
sapiens (***Human***), 154 . . . 497 168/355 (46%)
569 aa.
DETD . . . 251 250/297 e-142
associated protein (84%)
#109--Mammalia, 1 . . . 297 250/297
297 na. (84%)

	[WO200230268-A2, 18 APR. 2002]				
AAB88388	***Human*** membrane or secretory protein clone PSEC0131-- Homo sapiens, 297 aa. [EP1067182-A2, 10 JAN. 2001]	1 . . . 251 1 . . . 297	250/297 (84%) 250/297 (84%)		e-142
AAE21272	***Human*** gene 16 encoded secreted protein fragment, SEQ ID NO: 138-- Homo sapiens, 207 aa. [WO200216390-A1, 28 FEB. 2002]	92 . . . 251 2 . . . 207	159/206 (77%) 159/206 (77%)		4e-85
ABG64865	***Human*** albumin fusion protein #1540--Homo sapiens, 115 aa. [WO200177137-A1, 18 OCT. 2001]	173 . . . 251 37 . . . 115	79/79 (100%) 79/79 (100%)		4e-41
ABB90241	***Human*** polypeptide SEQ ID NO 2617-- Homo sapiens,	173 . . . 251 37	79/79 (100%)		4e-41
DETD . . .	1 . . . 251	250/297 (84%)	e-142		
	from Patent EP1067182-- Homo sapiens (***Human***), 297 aa.	1 . . . 297	250/297 (84%)		
AAH29530	Similar to RIKEN cDNA 2810417M05 gene--Homo sapiens (***Human***), 297 aa.	1 . . . 251 1 . . . 297	249/297 (83%) 250/297 (83%)		e-142
Q9CZ16	2810417M05Rik protein--Mus musculus	1 . . . 194 1 . . . 240	188/240 (78%) 191/240 (79%)		e-104
. . .	23/60 (38%)	0.43			
	fis, clone NT2RP3004481, weakly similar to BUTYROPHILIN PRECURSOR-- Homo sapiens (***Human***), 388 aa.	323 . . . 376	31/60 (51%)		
CAC35426	Sequence 1 from Patent WO0118204-- Homo sapiens (***Human***), 340 aa.	10 . . . 64 275 . . . 328	23/60 (38%) 31/60 (51%)		0.43
DETD . . .	for NOV38a				

Geneseq Identifier	Protein/ Organism/Length [Patent #, Date]	NOV38a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAU70673	***Human*** otoferlin #2--Homo sapiens, 1997 aa. [WO200170972-A2, Sep. 27, 2001]	1 . . . 1984 1 . . . 1994 1978 1 . . . 1983	811/2104 (38%) 1198/2104 (56%) 797/2091 (38%)	0.0 0.0
	#2--Mus sp, 1992 aa.		1188/2091	
AAU70675	***Human*** otoferlin--Homo	803 . . . 1984	540/1249 (43%)	0.0

	sapiens, 1230 aa. [WO200170972-A2, 27 SEP. 2001]	12 . . . 1227	769/1249 (61%)	
AAU70672	***Human*** otoferlin #1--Homo sapiens, 1307 aa. [WO200170972-. . .	783 . . . 1984 49 . . . 1304	542/1289 (42%) 778/1289	0.0

DETD . . . Matched Expect

Number	Organism/Length	Residues	Portion	Value
Q9HC10	Otoferlin (Fer-1 like protein 2)--Homo sapiens (***Human***), 1997 aa.	1 . . . 1984 1 . . . 1994	810/2104 (38%) 1197/2104 (56%)	0.0
Q9ESF1	Otoferlin (Fer-1 like protein. . . (100%) protein similar to otoferlin (A FER-1-like protein))--Homo sapiens (***Human***), 615 aa (fragment).	1 . . . 1984 1 . . . 615	803/2100 615/615 (100%)	0.0
Q9NTZ8	DJ309K20.1.1 (Novel protein similar to dysferlin, isoform 1)--Homo sapiens (***Human***), 552 aa (fragment).	231 . . . 782 1 . . . 552	552/552 (100%) 552/552 (100%)	0.0
Q9H448	DJ477O4.1.1 (Novel protein similar to otoferlin and dysferlin, isoform 1)--Homo sapiens (***Human***), 531 aa (fragment).	1500 . . . 1990 1 . . . 531	491/531 (92%) 491/531 (92%)	0.0

DETD . . . NOV39a

Geneseq Identifier	Protein/ Organism/Length [Patent #, Date]	NOV39a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABG13799	Novel ***human*** diagnostic protein #13790--Homo sapiens, 681. . . BFIV19v1 class I MHC protein--Gallus gallus, 338 aa. [US6075125-A, 13 JUN. 2000]	35 . . . 196 518 . . . 680 97 . . . 309	93/163 (57%) 118/163 (72%) 105/221 (47%)	5e-49
AAG00593	***Human*** secreted protein, SEQ ID NO:. . .	1 . . . 64 1 . . . 64	39/64 (60%) 46/64 (70%)	1e-15

DETD 333 333/333 (100%) 0.0

	glycoprotein CD1b precursor (CD1b antigen)--Homo sapiens (***Human***), 333 aa.	1 . . . 333	333/333 (100%)
Q28565	T-cell surface glycoprotein	1 . . . 332 1 . . . 332	248/332 (74%) 280/332 (83%)

. . .

DETD . . . Results for NOV40a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV40a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect

Value

AAB10284 ***Human*** fetal placenta protein 1 . . . 148 148/173 (85%) 5e-82
fragment AC175_2i - Homo 1 . . . 173 148/173 (85%)
sapiens, 173 aa.
[WO200037630-A1,
Jun. 29, 2000]
AAG03464 ***Human*** secreted protein, SEQ 1 . . . 76 76/91 (83%) 5e-37
ID NO: 7545 - Homo 1 . . . 91 76/91 (83%)
sapiens, 91 aa.
[EP1033401-A2,
Sep. 6, 2000]
ABP41833 ***Human*** ovarian antigen 58 . . . 116 33/59 (55%) 4e-17
HOPJF55, SEQ ID NO:2965 - 145 . . . 203 48/59 (80%)
Homo sapiens, 232 aa.
[WO200200677-A1,
Jan. 3, 2002]
AAU30569 Novel ***human*** secreted 58 . . . 115 32/58 (55%) 2e-16
protein #1060 - Homo 114 . . . 171 47/58 (80%)
. . .
DETD . . . protein 5 precursor (MFAP-5) 1 . . . 173 148/173 (85%)
(Microfibril- associated
glycoprotein 2) (MAGP-2)
(MP25) - Homo sapiens
(***Human***), 173 aa.
Q28022 Microfibrillar-associated 1 . . . 148 118/170 (69%) 2e-64
protein 5 precursor (MFAP-5) 1 . . . 170. . .
DETD . . . e-169
protein designated BMS53 - 2 . . . 465 362/471 (76%)
Homo sapiens, 466 aa.
[WO9933979-A2,
Jul. 8, 1999]
ABB89128 ***Human*** polypeptide SEQ ID 338 . . . 607 266/270 (98%) e-150
NO 1504 - Homo sapiens, 1 . . .. . . SEQ ID NO 2140 . . . 2740 366/607 (59%)
18432 - Drosophila
melanogaster, 2771 aa.
[WO200171042-A2,
Sep. 27, 2001]
AAB56086 ***Human*** secreted protein 246 . . . 605 201/366 (54%) e-109
sequence encoded by gene 10 20 . . . 378 261/366 (70%)
SEQ ID NO:180 - Homo
sapiens, 379 aa.
[WO200070042-A1,
Nov. 23, 2000]
ABB89513 ***Human*** polypeptide SEQ ID 48 . . . 180 97/133 (72%) 2e-48
NO 1889 - Homo sapiens, 1 . . .. . .
DETD . . . clone 1 . . . 607 533/607 (87%) 0.0
HEP18857 - Homo sapiens 1 . . . 544 537/607 (87%)
(***Human***), 544 aa.
Q92508 Hypothetical protein 10 . . . 605 381/602 (63%) 0.0
KIAA0233 - Homo sapiens 1440 . . . 2034 467/602 (77%)
(***Human***), 2035 aa.
Q9VLS3 CG8486 protein - Drosophila 28 . . . 605 233/607 (38%) e-124
melanogaster (Fruit fly), 2771 2140 .. . . clone 423 . . . 572 150/150 (100%) 2e-81
LNG09262 - Homo sapiens 1 . . . 150 150/150 (100%)
(***Human***), 150 aa.
DETD . . . Results for NOV42a

	NOV42a Residues/	Identities/ Similarities for

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	Match Residues	the Matched Region	Expect Value
AAU00782	***Human*** apoptosis protein, APOP-2 - Homo sapiens, 311 aa. [WO200118042-A2, Mar. 15, 2001]	100 . . . 392 19 . . . 311	291/293 293/293 (99%)	(99%) e-179
ABB90335	***Human*** polypeptide SEQ ID NO 2711 - Homo sapiens, 296 aa. [WO200190304-A2, Nov. 29, 2001]	15 . . . 293 7 . . . 284	183/279 230/279 (81%)	(65%) e-116
AAB93884	***Human*** protein sequence SEQ ID NO:13813 - Homo sapiens, 394 aa. [EP1074617-A2, Feb. 7, 2001]	15 . . . 361 8 . . . 353	160/347 218/347 (62%)	(46%) 4e-90
ABB90167	***Human*** polypeptide SEQ ID NO 2543 - Homo sapiens, 394 aa. [WO200190304-A2, Nov. 29, 2001]	15 . . . 360 8 . . . 352	159/346 217/346 (61%)	(45%) 1e-89
AAM78909	***Human*** protein SEQ ID NO 1571 - Homo sapiens, 394 aa.	15 . . . 360 8	159/346	(45%) 1e-89
DETD . . .	1 . . . 392 391/392 (99%) 0.0			
	2310081H14 gene - Homo sapiens (***Human***), 392 aa.	1 . . . 392	391/392 (99%)	
Q924Z3	TRH4 - Mus musculus (Mouse), 414 aa.	1 . . . 392 1	301/392 (76%)	0.0
DETD . . .	for NOV43a			

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV43a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABG14568	Novel ***human*** diagnostic protein #14559 - Homo sapiens, 363 aa. [WO200175067-A2, Oct. 11, 2001]	1 . . . 332 1 . . . 333	325/333 326/333 (97%)	(97%) 0.0
AAB82940	***Human*** androgen receptor trapped protein 5 (ARTS) - Homo sapiens, 264 aa. [WO200172332-A1, Oct. 4, 2001]	392 . . . 550 94 . . . 253	63/161 98/161 (60%)	(39%) 2e-26
AAB56085	***Human*** secreted protein sequence encoded by gene 9 SEQ ID NO:179 - Homo sapiens, 264 aa. [WO200070042-A1, Nov. 23, 2000]	392 . . . 550 94 . . . 253	63/161 98/161 (60%)	(39%) 2e-26
AAW76212	***Human*** ELL2 protein - Homo sapiens, 640 aa. [WO9837194-A1, Aug. 27, 1998]	371 . . . 551 466 . . . 633	60/184 100/184 (53%)	(32%) 6e-19
AAB57048	***Human*** prostate cancer antigen protein sequence	371 . . . 551 503 . . . 670	60/184 99/184 (53%)	(32%) 1e-18

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DETD   .  .  .  1 . . . 561  557/561 (99%)    0.0
             protein FLJ30532 - Homo          1 . . . 558  557/561 (99%)
             sapiens (  ***Human***  ), 558 aa.
Q96NM9       CDNA FLJ30532 fis, clone         1 . . . 433  429/433 (99%)
       0.0
             BRAWH2001129, weakly             1 . . . 430  429/433 (99%)
             similar to occludin - Homo
             sapiens (  ***Human***  ), 457 aa.
Q99LE8       Hypothetical 50.4 kDa           121 . . . 560 386/441 (87%)
       0.0
             protein - Mus musculus           1 . ..  .  .  clone       392 .
       . . 550  63/161 (39%)    5e-26
             HSI13338 - Homo sapiens          94 . . . 253  98/161 (60%)
             (  ***Human***  ), 264 aa.
Q8VCR9       Similar to RIKEN cDNA           437 . . . 550  49/114 (42%)
       2e-20
             9430098E02 gene - Mus            94 ..  .  .
DETD   .  .  .  Results for NOV44a
                                                 NOV44a      Identities/
                                                 Residues/   Similarities for
Geneseq      Protein/Organism/Length             Match       the Matched
       Expect
Identifier   [Patent #, Date]                    Residues    Region
       Value

AAB62698       ***Human***   membrane recycling              1 . . . 306
       306/347 (88%)    e-173
             protein (HMRP)-1 - Homo             1 . . . 347 306/347 (88%)
             sapiens, 347 aa.
             [U.S. Pat. No. 6,235,715-B1,
             May 22, 2001]
AAY30521     A   ***human***   membrane                      1 . . . 306
       306/347 (88%)    e-173
             recycling protein designated        1 . . . 347 306/347 (88%)
             HMRP-1.  .  .  178/333 (53%)   3e-90
             protein #122 - Mammalia,            7 . . . 334 215/333 (64%)
             338 aa. [WO200230268-A2,
             Apr. 18, 2002]
AAB62699       ***Human***   membrane recycling              9 . . . 298
       164/322 (50%)   1e-87
             protein (HMRP)-2 - Homo             7 . . . 325 220/322.  .  .
DETD   .  .  .  . . . 306   302/347 (87%)   e-170
             membrane protein 3 - Homo     1 . . . 347   302/347 (87%)
             sapiens (  ***Human***  ), 347 aa.
T08826       secretory carrier membrane    1 . . . 306   301/347 (86%)   e-169
             protein homolog propin1 -     1 . . . 347   301/347 (86%)
                 ***human***  , 347 aa.
Q99M48       Similar to secretory carrier  1 . . . 306   277/350 (79%)   e-156
             membrane protein 3 - Mus      1.  .  .
DETD   .  .  .  for NOV45a
                                           NOV45a          Identities/
                                           Residues/       Similarities for
Geneseq      Protein/Organism/Length       Match           the Matched
       Expect
Identifier   [Patent #, Date]              Residues        Region
       Value

AAB07747     A   ***human***   cancer-associated      33 . . . 319   148/287
     . (51%)    7e-89
             protein-1 (CAP-1) - Homo      178 . . . 464   205/287 (70%)
.  .  .
DETD   .  .  .  . . . 321 289/289 (100%)        e-168
             protein SRPX precursor -      176 . . . 464 289/289 (100%)
             Homo sapiens (  ***Human***  ), 464
             aa.
Q63769       Sushi repeat-containing        33 . . . 321 279/289 (96%)
       e-164
             protein SRPX precursor        176 . . . 464.  .  .
DETD   .  .  .  Results for NOV46a
                                           NOV46a
                                           Residues/     Identities/
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Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	Match Residues	Similarities for the Matched Region	Expect Value
AAE15253	***Human*** RNA metabolism protein-16 (RMEP-16) - Homo sapiens, 319 aa. [WO200183524-A2, Nov. 8, 2001]	19 . . . 239 99 . . . 319	221/221 221/221 (100%)	e-131 e-131 (100%)
AAM78405	***Human*** protein SEQ ID NO 1067 - Homo sapiens, 319 aa. [WO200157190-A2, Aug. 9, 2001]	19 . . . 239 99 . . . 319	221/221 221/221 (100%)	e-131 e-131 (100%)
AAM79389	***Human*** protein SEQ ID NO 3035 - Homo sapiens, 354 aa. [WO200157190-A2, Aug. 9, 2001]	19 . . . 236 137 . . . 354	215/218 216/218 (98%)	e-127 e-127 (98%)
ABB11888	***Human*** novel protein, SEQ ID NO:2258 - Homo sapiens,	19 . . . 236 137	215/218 . .	e-127 (98%)

DETD Number	Protein/Organism/Length	Residues	Matched Portion	Expect Value
P57060	Protein C21orf6 (GL011) - Homo sapiens (***Human***), 319 aa.	19 . . . 239 99 . . . 319	221/221 (100%) 221/221 (100%)	e-130
Q99M03	Similar to open reading frame	21 . . . 239	182/219. . .	

DETD . . . aa MW at 64468.4 kD

NOV47c, CG51595-04 Protein Sequence

CNGDCGAVRTRKRTLVGKSKKKEKCKNSHLYPLIETQYCPCDKYNAQPVGNWSDCI LPEGKVEVLLG

MKVQGDIKECGQGYRYQAMACYDQNGRLVETSRCNSHGYIEEACIIPCPSDCKLSE WSNWSRCSKSC

GSGVKVRSKWLREKPYNGGRPCPKLDHVNQAQVYEVVPCHSDCNQYLWVTEPWSIC KVTFVNMRENC

GEGVQTRKVRCMQNTADGPSEHVEDYLCDPEEMPLGSRVCKLPCPEDCVISEWGPW TQCVLPCNQSS

FRQRSADPIRQPADEGRSCPNAVEKEPCNLNKNCYHYDYNVTDWSTCQLSEKAVCG NGIKTRMLDCV

RSDGKSVDLKYCEALGLEKNWQMNTSCMVECPVNCQLSDWSPWSECSQTCGLTGKM IRRRTVTQPFQ

GDGRPCPSLMDQSKPCPVKPCYRWQYGQWSPCQVQEAQCGEGTRTRNISCVVSDGS ADDFSKVVDEE

FCADIELIIDGNKNMVLEESCSQPCPGDCYLKDWSSWSLCQLTCVNGEDLG *** FGGIQVRSRPVIIQEL***

ENQHLCPEQMLETKSCYDGQCYEYKWMASAWKGSS

SEQ ID NO: 181 14881 bp

NOV47d, CG51595-06 DNA Sequence

CGTCCATGGGGCCGATGTATGGGAGATGAATGTGGTCCCGGAGGCATCCAAACGAG GGCTGTGTGGT

GTGCTCATGTGGAGGGATGGACTACACTGCATACTAACTGTAAGCAGGCCGAGAGA CCCAATAACCA

GCAGAATTGTTTCAAAGTTTGCGATTGGCACAAAGAGTTGTACGACTGGAGACTGG GACCTTGGAAT

CAGTGTCAGCCCGTGATTTCAAAAAGCCTAGAGAAACCTCTTGAGTGCATTAAGGG
GGAAGAAGGTA

TTCAGGTGAGGGAGATAGCGTGCATCCAGAAAGACAAAGACATTCCTGCGGAGGAT
ATCATCTGTGA

. . .

DETD 1588 1588/1588 (100%) 0.0
Homo sapiens, 1588 aa. 1 . . . 1588 1588/1588 (100%)
[WO200105971-A2,
Jan. 25, 2001]
AAM39295 ***Human*** polypeptide SEQ ID 1 . . . 1588 1587/1588
(99%) 0.0
NO 2440 - Homo sapiens, 1 . . . 1588 1588/1588 (99%)
1588 aa.
[WO200153312-A1,
Jul. 26, 2001]
AAM41081 ***Human*** polypeptide SEQ ID 48 . . . 1588 1540/1541
(99%) 0.0
NO 6012 - Homo sapiens, 11 . . . 1551 1540/1541 (99%)
1551 aa.
[WO200153312-A1,
Jul. 26, 2001]
AA342496 ***Human*** ORFX ORF2260 1 . . . 614 605/614
(98%) 0.0
polypeptide sequence SEQ 6 . . . 617 607/614 (98%)

. . .

DETD . . . Patent 1 . . . 1588 1588/1588 (100%) 0.0
WO0105971 - Homo 1 . . . 1588 1588/1588 (100%)
sapiens (***Human***), 1588 aa.
BAA76804 KIAA0960 protein - Homo 87 . . . 1588 1502/1502 (100%)
0.0
sapiens (***Human***), 1502 aa 1 . . . 1502 1502/1502
(100%)
(fragment).
Q9UPZ6 KIAA0960 protein - Homo 299 . . . 1588 1290/1290 (100%)
0.0
sapiens (***Human***), 1290 aa 1 . . . 1290 1290/1290
(100%)
(fragment).
Q9C0I4 KIAA1679 protein - Homo 22 . . . 1588 790/1574 (50%)
0.0
sapiens (***Human***), 1536 aa 1 . . . 1536 1044/1574
(66%)
(fragment).
O43384 Hypothetical protein 954 . . . 1401 446/448 (99%)
0.0
GS164B05.1 in 1 . . . 446 446/448 (99%)
chromosome 7 - Homo
sapiens (***Human***), 446 aa
(fragment).

DETD . . . Results for NOV48a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV48a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAB71869	***Human*** EMR1 seven	1 . . . 886	886/886 (100%)	0.0
	transmembrane domain - Homo sapiens, 886 aa. [WO200109328-A1, Feb. 8, 2001]	1 . . . 886	886/886 (100%)	
AAB01249	***Human*** EMR1 hormone receptor - Homo sapiens, 880 aa. [WO200034473-A2, Jun. 15, 2000]	1 . . . 886	880/886 (99%)	0.0
		1 . . . 880	880/886 (99%)	
AAE17043	***Human*** CD 97 protein -	74 . . . 872	272/853 (31%)	e-122

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            Homo sapiens, 835 aa.          16 . . . 817  422/853 (48%)
            [WO200202602-A2,
            Jan. 10, 2002]
AAB15728      ***Human***   CD97 protein -          74 . . . 872  272/853
      (31%)       e-122
            Homo sapiens, 835 aa.          16 . . . 817  422/853 (48%)
            [WO200052039-A2,
            Sep. 8, 2000]
AAY41090      ***Human***   CD97 protein -          74 . . . 872  272/853
      (31%)       e-122
            Homo sapiens, 835 aa.          16 . . . 817.  . .
DETD  . . .  . 886  886/886 (100%)       0.0
            EMR1 precursor (EMR1            1 . . . 886  886/886 (100%)
            hormone receptor) - Homo
            sapiens (  ***Human***  ), 886 aa.
BAC06133    Seven transmembrane helix      11 . . . 885  866/877 (98%)
      0.0
            receptor - Homo sapiens        29 . . . 905  868/877 (98%)
            (  ***Human***  ), 929 aa.
Q61549      Cell surface glycoprotein       1 . . . 886  606/937 (64%)
      0.0
            EMR1 precursor (EMR1            1 . . . .  . . helix     74 . . .
      872  272/853 (31%)       e-121
            receptor - Homo sapiens        18 . . . 819  422/853 (48%)
            (  ***Human***  ), 837 aa.
O00718      CD97 - Homo sapiens            74 . . . 872  272/853 (31%)
      e-121
            (  ***Human***  ), 835 aa.              16 . . . 817  422/853
      (48%)
DETD  . . .  0.0
            gamma 3 chain--                          (100%)
            Mus musculus,           1 . . . 1587 1587/1587
            1587 aa.                             (100%)
            [WO200183516-A1,
            8 NOV. 2001]
AAB40917      ***Human***   ORFX              1 . . . 1587 1585/1587     0.0
            ORF681 poly-                         (99%)
            peptide sequence        1 . . . 1587 1586/1587
            SEQ ID NO:                           (99%)
            1362--Homo
            sapiens, 1587 aa.
            [WO200058473-A2,
            5 OCT. 2000]
AAY15458      ***Human***   laminin          67 . . . 1587 1493/1524     0.0
            gamma 3 subunit--                    (97%)
            Homo sapiens,           1 . . . 1524 1496/1524
            1524 aa.                             (97%)
            [WO9919348-A1,
            22 APR. 1999]
AAB19803      ***Human***   laminin 2        10 . . . 1583  698/1599     0.0
            gamma-1 chain with                   (43%)
            C-terminal FLAG         21 . . . 1600  964/1599
            epitope--Homo                        (59%)
            sapiens, 1617 aa.
            [WO200066730-A2,
            9 NOV. 2000]
AAB19801      ***Human***   laminin 2        10 . . . 1583  698/1599     0.0
            gamma-1 chain--                      (43%)
            Homo sapiens,           21 . . . 1600  964/1599
. . .
DETD  . . .  . . 1587 1587/1587     0.0
            chain precursor                      (100%)
            (Laminin 12 gamma       1 . . . 1587 1587/1587
            3)--Homo sapiens                     (100%)
            (  ***Human***  ), 1587 aa.
Q9R0B6      Laminin gamma-3         17 . . . 1585 1169/1572     0.0
            chain precursor                      (74%)
            (Laminin 12 gamma       26 . . .  . . 25 from     10 . . . 1583
      698/1599     0.0
            Patent WO0066730                     (43%)
            precursor--Homo         21 . . . 1600  964/1599
```

	sapiens (***Human***), 1617 aa.			(59%)	
CAC17323	Sequence 21 from Patent WO0066730	10 . . . 1583	698/1599 (43%)	0.0	
	precursor--Homo sapiens (***Human***), 1609 aa.	21 . . . 1600	964/1599 (59%)		
P11047	Laminin gamma-1 chain precursor (Laminin B2 chain)--Homo sapiens (***Human***), 1609 aa.	10 . . . 1583	697/1599 (43%)	0.0	
		21 . . . 1600	963/1599 (59%)		
DETD . . . for NOV50a					

Geneseq Identifier	Protein/ Organism/Length [Patent #, Date]	NOV50a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
ABB90211	***Human*** polypeptide SEQ ID NO 2587--Homo sapiens, 324 aa. [WO200190304-A2, 29 NOV. 2001]	1 . . . 311 14 . . . 324	310/311 (99%) 310/311 (99%)	e-176
AAB51239	***Human*** hTMPT27 protein sequence SEQ ID NO: 7--Homo sapiens, 324 aa. [CN1268567-A, 4 OCT. 2000]	1 . . . 311 14 . . . 324	310/311 (99%) 310/311 (99%)	e-176
AAB20092	***Human*** hydrophobic domain-containing protein HP03373--Homo sapiens, 324 aa. [WO200100824-A2, 4 JAN. 2001]	1 . . . 311 14 . . . 324	310/311 (99%) 310/311 (99%)	e-176
AAB41971	***Human*** ORFX ORF1735 polypeptide sequence	1 . . . 311 12 . . . 322	310/311 (99%) 310/311 (99%)	e-176
. . .				
DETD . . . Transmembrane protein PT27--Homo sapiens (***Human***), 324 aa.		1 . . . 311 14 . . . 324	310/311 (99%) 310/311 (99%)	e-176
Q9NZ34	Uncharacterized hypothalamus protein HTMP--Homo sapiens (***Human***), 324 aa.	1 . . . 311 14 . . . 324	310/311 (99%) 310/311 (99%)	e-176
Q9R292	TPARDL--Mus musculus	12 . . . 311 15 . . . 323	287/310 (92%) 293/310 (93%)	e-161
. . .				

DETD . . . expression analysis by transcript profiling coupled to a gene database query" Nature Biotechnology 17:198-803 (1999). cDNA was derived from various ***human*** samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained. . .

DETD [0642] 2. SeqCalling.TM. Technology: cDNA was derived from various ***human*** samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained. . . were evaluated manually and edited for corrections if appropriate. cDNA sequences from all samples were assembled together, sometimes including public ***human*** sequences, using bioinformatic programs to produce a consensus sequence for each assembly. Each assembly is included in CuraGen Corporation's database.. . .

DETD . . . are sequenced. In silico prediction was based on sequences

available in CuraGen Corporation's proprietary sequence databases or in the public ***human*** sequence databases, and provided either the full length DNA sequence, or some portion thereof.

DETD [0645] cDNA libraries were derived from various ***human*** samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained. . .

DETD [0646] Gal4-binding domain (Gal4-BD) fusions of a CuraGen Corportion proprietary library of ***human*** sequences was used to screen multiple Gal4-AD fusion cDNA libraries resulting in the selection of yeast hybrid diploids in each. . . were evaluated manually and edited for corrections if appropriate. cDNA sequences from all samples were assembled together, sometimes including public ***human*** sequences, using bioinformatic programs to produce a consensus sequence for each assembly. Each assembly is included in CuraGen Corporation's database.. . .

DETD . . . or complete the predicted sequence of the cDNA of the invention. Usually multiple clones were sequenced from one or more ***human*** samples to derive the sequences for fragments. Various ***human*** tissue samples from different donors were used for the RACE reaction. The sequences derived from these procedures were included in. . .

DETD . . . the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related ***human*** sequences from other species. These primers were then employed in PCR amplification based on the following pool of ***human*** cDNAs: adrenal gland, bone marrow, brain--amygdala, brain--cerebellum, brain--hippocampus, brain--substantia nigra, brain--thalamus, brain--whole, fetal brain, fetal kidney, fetal liver, fetal lung,. . .

DETD . . . lines), Panel 4 (containing cells and cell lines from normal tissues and cells related to inflammatory conditions), Panel 5D/51 (containing ***human*** tissues and cell lines with an emphasis on metabolic diseases), AI_comprehensive_panel (containing normal tissue and samples from autoinflammatory diseases), Panel. . .

DETD . . . 2.2, 2.3 and 2.4 generally include 2 control wells and 94 test samples composed of RNA or cDNA isolated from ***human*** tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative ***Human*** Tissue Network (CHTN) or the National Disease Research Initiative (NDRI) or from Ardais or Clinomics). The tissues are derived from ***human*** malignancies and in cases where indicated many malignant tissues have "matched margins" obtained from noncancerous tissue just adjacent to the. . . surgery (designated "NAT", for normal adjacent tissue, in Table RR). In addition, RNA and cDNA samples were obtained from various ***human*** tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissues were ascertained to be. . .

DETD . . . 1.0 plates are comprised of 93 cDNA samples and two controls. Specifically, 81 of these samples are derived from cultured ***human*** cancer cell lines that had been subjected to serum starvation, acidosis and anoxia for different time periods as well as controls for these treatments, 3 samples of ***human*** primary cells, 9 samples of malignant brain cancer (4 medulloblastomas and 5 glioblastomas) and 2 controls. The ***human*** cancer cell lines are obtained from ATCC (American Type Culture Collection) and fall into the following tissue groups: breast cancer,. . . recommended conditions. The treatments used (serum starvation, acidosis and anoxia) have been previously published in the scientific literature. The primary ***human*** cells were obtained from Clonetics (Walkersville, Md.) and were grown in the media and conditions recommended by Clonetics. The malignant. . .

DETD . . . plates for ARDAIS panel v 1.0 generally include 2 control wells and 22 test samples composed of RNA isolated from ***human*** tissue procured by surgeons working in close cooperation with Ardais Corporation. The tissues are derived from ***human*** lung malignancies (lung adenocarcinoma or lung squamous cell carcinoma) and in cases where indicated many malignant samples have "matched margins". . .

DETD . . . 3.2 are comprised of 94 cDNA samples and two control samples. Specifically, 92 of these samples are derived from cultured

human cancer cell lines, 2 samples of ***human*** primary cerebellar tissue and 2 controls. The ***human*** cell lines are generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall. . .

DETD . . . well plate (2 control wells, 94 test samples) composed of RNA (Panel 4R) or cDNA (Panels 4D/4.1D) isolated from various ***human*** cell lines or tissues related to inflammatory conditions. Total RNA from control normal tissues such as colon and lung (Stratagene,. . .

DETD . . . dermal fibroblasts, coronary artery smooth muscle cells, small airway epithelium, bronchial epithelium, microvascular dermal endothelial cells, microvascular lung endothelial cells, ***human*** pulmonary aortic endothelial cells, ***human*** umbilical vein endothelial cells were all purchased from Clonetics (Walkersville, Md.) and grown in the media supplied for these cell. . .

DETD . . . blood of employees at CuraGen Corporation, using Ficoll. LAK cells were prepared from these cells by culture in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco/Life Technologies, Rockville, Md.), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and. . . IL-18 at 5-10 ng/ml for 6 hours. In some cases, mononuclear cells were cultured for 4-5 days in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . . cells using Ficoll and mixing the isolated mononuclear cells 1:1 at a final concentration of approximately 2.times.10.sup.6cells/ml in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol (5.5.times.10.sup.-5M) (Gibco), and 10 mM Hepes. . .

DETD . . . Magnet according to the manufacturer's instructions. Monocytes were differentiated into dendritic cells by culture in DMEM 5% fetal calf serum (***FCS***) (Hyclone, Logan, Utah), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10. . . and 5 ng/ml IL-4 for 5-7 days. Macrophages were prepared by culture of monocytes for 5-7 days in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), 10 mM Hepes (Gibco) and 10% AB ***Human*** Serum or MCSF at approximately 50 ng/ml. Monocytes, macrophages and dendritic cells were stimulated for 6 and 12-14 hours with. . .

DETD . . . with the remaining cells being CD45RA CD4 lymphocytes. CD45RA CD4, CD45RO CD4 and CD8 lymphocytes were placed in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . . for 4 days on anti-CD28 and anti-CD3 coated plates and then harvested the cells and expanded them in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10M (Gibco), and 10 mM Hepes. . . second activation and after 4 days of the second expansion culture. The isolated NK cells were cultured in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . .

DETD . . . scissors and then passed through a sieve. Tonsil cells were then spun down and resupended at 10.sup.6cells/ml in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . .

DETD . . . twice with PBS. Umbilical cord blood CD4 lymphocytes (Poietic Systems, German Town, Md.) were cultured at 10.sup.5-10.sup.6cells/ml in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), 10 mM Hepes (Gibco). . . the activated Th1, Th2 and Tr1 lymphocytes were washed once in DMEM and expanded for 4-7 days in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.5M (Gibco), 10 mM Hepes (Gibco). . .

DETD . . . the culture of these cells, we used DMEM or RPMI (as recommended by the ATCC), with the addition of 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), 10 mM

Hepes (Gibco).. . . CCD106 and an airway epithelial tumor line NCI-H292 were also obtained from the ATCC. Both were cultured in DMEM 5% ***FCS*** (Hyclone), 100 .mu.M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5.times.10.sup.-5M (Gibco), and 10 mM Hepes. . .

DETD . . . plates for AI_comprehensive panel_v1.0 include two control wells and 89 test samples comprised of cDNA isolated from surgical and postmortem ***human*** tissues obtained from the Backus Hospital and Clinomics (Frederick, Md.). Total RNA was extracted from tissue samples from the Backus. . .

DETD [0713] The plates for Panel 5D and 5I include two control wells and a variety of cDNAs isolated from ***human*** tissues and cell lines with an emphasis on metabolic diseases. Metabolic tissues were obtained from patients enrolled in the Gestational Diabetes study. Cells were obtained during different stages in the differentiation of adipocytes from ***human*** mesenchymal stem cells. ***Human*** pancreatic islets were also obtained.

DETD . . . of Clonetics/BioWhittaker) in triplicate, except for Donor 3U which had only two replicates. Scientists at Clonetics isolated, grew and differentiated ***human*** mesenchymal stem cells (HuMSCs) for CuraGen based on the published protocol found in Mark F. Pittenger, et al., Multilineage Potential of Adult ***Human*** Mesenchymal Stem Cells Science Apr. 2, 1999: 143-147. Clonetics provided Trizol lysates or frozen pellets suitable for mRNA isolation and. . .

DETD [0724] ***Human*** cell lines were generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall. . .

DETD [0735] The plates for Panel CNSD.01 include two control wells and 94 test samples comprised of cDNA isolated from postmortem ***human*** brain tissue obtained from the Harvard Brain Tissue Resource Center. Brains are removed from calvaria of donors between 4 and. . .

DETD [0745] The plates for Panel CNS_Neurodegeneration_V1.0 include two control wells and 47 test samples comprised of cDNA isolated from postmortem ***human*** brain tissue obtained from the Harvard Brain Tissue Resource Center (McLean Hospital) and the ***Human*** Brain and Spinal Fluid Resource Center (VA Greater Los Angeles Healthcare System). Brains are removed from calvaria of donors between. . .

DETD . . . and macrophages. This gene encodes a protein with homology to BLAST1, an activation-associated cell surface glycoprotein expressed primarily in mitogen-stimulated ***human*** lymphocytes. The expression of this gene in hematopoietic cells and thymus on Panel 1.4 is consistent with this characterization. Highest. . .

DETD . . . activated-NCI-H292 mucoepidermoid cells as well as untreated NCI-H292 cells, IL-4, IL-9, IL-13 and IFN gamma activated lung and dermal fibroblasts, ***human*** pulmonary aortic endothelial cells (treated and untreated), small airway epithelium (treated and untreated), treated bronchial epithelium and lung and dermal. . .

DETD . . . NCI-H292 cell line (CT=30.4). This gene is also expressed in a cluster of treated and untreated NCI-H292 cell line, a ***human*** airway epithelial cell line that produces mucins. Mucus overproduction is an important feature of bronchial asthma and chronic obstructive pulmonary. . .

DETD . . . this panel. In addition, low levels of expression of this gene are also seen in cytokine activated NCI-H292 cells, a ***human*** airway epithelial cell line that produces mucins. Therefore, modulation of the expression or activity of the protein encoded by this. . .

DETD . . . fetal liver. These tissues may contain monocytes or monocytic derived cell types. This gene codes for EMR1 hormone receptor precursor (***human*** F4/80 homologue). EMR1 is a member of the family of hormone receptors with seven transmembrane segments. In addition, it has. . .

DETD . . . all or part of the initial or extended sequence were identified by BLASTN searches using the relevant sequence to query ***human*** genomic databases. The genomic clones that resulted were selected for further analysis because this identity indicates that these clones contain. . .

DETD . . . identified was manually assembled and then may have been extended using one or more additional sequences taken from CuraGen Corporation's ***human*** SeqCalling database. SeqCalling fragments suitable for inclusion were identified by the CuraTools.TM. program SeqExtend or by identifying SeqCalling fragments mapping. . .

CLM What is claimed is:
8. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a ***human*** disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein the therapeutic comprises the polypeptide. . .
18. The method of claim 17, wherein the subject is a ***human*** .

L13 ANSWER 4 OF 24 USPATFULL on STN
AN 2004:44535 USPATFULL
TI Methods of using a three-dimensional model of a ***Fc*** ***epsilon*** ***receptor*** alpha chain
IN Jardetzky, Theodore S., Chicago, IL, UNITED STATES
Garman, Scott Clayton, Evanston, IL, UNITED STATES
Kinet, Jean-Pierre, Lexington, MA, UNITED STATES
PI US 2004033527 A1 20040219
AI US 2002-293992 A1 20021113 (10)
RLI Division of Ser. No. US 1999-434193, filed on 4 Nov 1999, PENDING
PRAI US 1998-107219P 19981105 (60)
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 84
ECL Exemplary Claim: 1
DRWN 8 Drawing Page(s)
LN.CNT 34663
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes three-dimensional models of antibody receptor proteins, such as ***Fc*** .epsilon.RI.alpha. proteins, and methods to produce such models. The present invention also includes muteins having increased stability and/or antibody binding activity, as well as methods to produce such muteins, preferably using information derived from three-dimensional models of the present invention. Also included are nucleic acid sequences encoding muteins of the present invention and use of those sequences to produce such muteins. Also included is the use of the model to identify compounds that inhibit the binding of an antibody receptor protein to an antibody. The present invention also includes uses of such muteins and inhibitory compounds, for example, in methods to diagnose and protect animals from allergy and other abnormal immune responses.
TI Methods of using a three-dimensional model of a ***Fc*** ***epsilon*** ***receptor*** alpha chain
AB The present invention includes three-dimensional models of antibody receptor proteins, such as ***Fc*** .epsilon.RI.alpha. proteins, and methods to produce such models. The present invention also includes muteins having increased stability and/or antibody binding activity,. . .
SUMM [0003] The present invention relates to a crystal and a three-dimensional (3-D) model of a ***Fc*** ***epsilon*** ***receptor*** alpha chain as well as to the use of that model to produce muteins and inhibitors useful in the diagnosis. . .
SUMM [0004] Antibody ***Fc*** -receptors (***FcRs***) play an important role in the immune response by coupling the specificity of secreted antibodies to a variety of cells of the immune system. A number of cell types, including macrophages, mast cells, eosinophils, and basophils, express membrane-bound ***FcRs*** at their surfaces. The binding of antibodies to ***FcRs*** provides antigen-specificity to these cells, which upon activation release further cell-specific mediators of the immune response, such as interleukins, initiators of inflammation, leukotrienes, prostaglandins, histamines, or cytotoxic proteins. The adoptive specificity of the ***FcRs*** allows a combinatorial approach to pathogen elimination, by coupling the diversity of antibody antigen-recognition sites to the variety of cell-types. . .
SUMM [0005] ***FcR*** -initiated mechanisms are important in normal immunity to infectious disease as well as in allergies, antibody-mediated tumor recognition, autoimmune diseases, and. . . other diseases in which immune responses are abnormal (i.e., not regulated). Recent experiments with transgenic mice have demonstrated that the ***FcRs*** control key steps in the immune response,

including antibody-directed cellular cytotoxicity and inflammatory cascades associated with the formation of immune complexes; see, for example, Ravetch et al., 1998, Annu Rev Immunolo 16, 421-432. Receptors that bind IgG (***FcgRI*** , ***FcgRII*** , and ***FcgRIII*** , known collectively as ***FcgRs***) mediate a variety of inflammatory reactions, regulate B-cell activation, and also trigger hypersensitivity reactions. The high affinity ***Fc*** ***epsilon*** ***receptor*** (also known as the IgE receptor or ***FceRI***) is associated with the activation of mast cells and the triggering of allergic reactions and anaphylactic shock. Knockout mice for the ***FceRI*** alpha chain (***Fc*** .epsilon.RI.alpha.) are unable to mount IgE-mediated anaphylaxis (see for example, Dombrowicz et al., 1993, Cell 75, 969-976), although ***FcgRs*** are still able to activate mast cells (see, for example, Dombrowicz et al., 1997, J. Clin. Invest. 99, 915-925; Oettgen et al., 1994, Nature 370, 367-370). ***FceRI*** has also been shown to trigger anti-parasitic reactions from platelets and eosinophils as well as deliver antigen into the MHC. . . Joseph et al., 1997, Eur. J. Immunol. 27, 2212-2218; Maurer et al., 1998, J. Immunol. 161, 2731-2739. The b-subunit of ***FceRI*** has been associated with asthma in genetic studies; see, for example, Hill et al., 1996, Hum. Mol. Genet. 5, 959-962;. . . (.about.20%) may be affected by allergies, and this century has seen a substantial increase in asthma. Since IgE binding to ***FceRI*** is a requisite event in the reaction to different allergens, therapeutic strategies aimed at inhibiting ***FceRI*** could provide a useful treatment for these diseases. For example, monoclonal antibodies that target IgE and block receptor binding have. . .

SUMM [0006] ***FceRI*** is found as a tetrameric (abg.sub.2) or trimeric (ag.sub.2) membrane bound receptor on the surface of mast cells, basophils, eosinophils, langerhans cells and platelets. The alpha chain, also referred to as ***Fc*** .epsilon.RI.alpha., of ***FceRI*** binds IgE molecules with high affinity (K.sub.D of about 10.sup.-9 to 10.sup.-10 moles/liter (M)), and can be secreted as a. . . 266, 2639-2646, which describes the secretion of a soluble IgE-binding fragment of 172 amino acids. The extracellular domains of the ***human*** ***Fc*** .epsilon.RI.alpha. protein belong to the immunoglobulin (Ig) superfamily and contain seven N-linked glycosylation sites. Glycosylation of ***Fc*** .epsilon.RI.alpha. affects the secretion and stability of the receptor, but is not required for IgE-binding; see, for example, LaCroix et al.,. . . 1993, J. Biol. Chem. 268, 12736-12743; Scarselli et al., 1993, FEBS Lett 329, 223-226. The beta and gamma chains of ***FceRI*** are signal transduction modules.

SUMM [0007] Prior investigators have disclosed the nucleic acid sequence for ***human*** ***Fc*** .epsilon.RI.alpha.; see, for example, U.S. Pat. No. 4,962,035, by Leder, issued Oct. 9, 1990; U.S. Pat. No. 5,639,660, by Kinet et. . . 85, 1907-1911; and Pang et al., 1993, J. Immunol. 151, 6166-6174. Nucleic acid sequences have also been reported for the ***human*** ***FC*** .epsilon.RI beta and gamma chains; see, respectively, Kuster et al., 1992, J. Biol. Chem. 267, 12782-12787; Kuster et al., 1990, J. Biol. Chem. 265, 6448-6452. Nucleic acid sequences have also been reported for nucleic acid molecules encoding canine ***Fc*** .epsilon.RI.alpha., murine ***Fc*** .epsilon.RI.alpha., rat ***Fc*** .epsilon.RI.alpha., feline ***Fc*** .epsilon.RI.alpha. and equine ***Fc*** .epsilon.RI.alpha. proteins; see, respectively, GenBank.TM. accession number D16413; Swiss-Prot accession number P20489 (represents encoded protein sequence); GenBank accession number J03606; PCT. . . al., published Aug. 5, 1999, referred to herein as WO 99/38974. In addition, methods to detect IgE antibodies using a ***Fc*** .epsilon.RI.alpha. protein have been reported in PCT Publication No. WO 98/23964, by Frank et al., published Jun. 4, 1998, referred to. . .

SUMM . . . have been several reports of the use of mutagenesis and swapping techniques to attempt to identify amino acids of either ***Fc*** .epsilon.RI.alpha. or IgE involved in the binding of (i.e., interaction between) those respective proteins, reports attempting to model ***Fc*** .epsilon.RI.alpha. proteins based on homology to other Ig-superfamily members, and reports that identify compounds that apparently inhibit such binding; see, for. . . No. WO 95/14779, by Gould et al., published Jun. 1, 1995. None of these references, however, describe isolated crystals of ***Fc*** .epsilon.RI.alpha. proteins or

3-D models derived from crystals.

SUMM [0009] Despite what is known about ***FcRs*** and their interaction with antibodies, there remains a need for ***FcRs*** with improved characteristics, such as enhanced affinity for antibodies, altered substrate specificity, increased stability, and increased solubility for use in. . .

SUMM [0010] The present invention includes isolated crystals of the extracellular domains of antibody receptor proteins (***FcRs***), three-dimensional (3-D) models of such crystals and modifications of such models. The present invention also includes compounds that inhibit the ability of ***FcRs*** to bind to antibodies as well as ***FcR*** muteins and other modified ***FcRs*** . Also included in the present invention are methods to produce and use such crystals, models, inhibitory compounds, muteins, and other modified proteins. As such, the present invention includes ***FcRs*** with improved functions such as increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, and increased solubility, including but not limited to reduced aggregation. Such proteins, also. . .

SUMM [0011] The present invention includes a 3-D model of an extracellular domain of a ***human*** high affinity ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.RI.alpha.) protein, wherein the model substantially represents the atomic coordinates specified in Table 1, Table 5, Table 6, Table 7 or. . .

SUMM [0012] The present invention also includes an isolated crystal of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and methods to produce such a crystal.

SUMM [0013] The present invention also includes an isolated ***Fc*** .epsilon.RI.alpha. protein consisting of SEQ ID NO: 2 or of SEQ ID NO: 4 except that the isoleucine at position 170. . .

SUMM . . . The present invention includes a method to identify a compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein. The method includes the step of using a 3-D model of an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein to identify the compound. Such a model substantially represents the atomic coordinates specified in Table 1, Table 5, Table. . .

SUMM [0015] The present invention also includes a mutein that binds to a ***Fc*** domain of an antibody. Such a mutein has an improved function compared to a protein that includes SEQ ID NO: 2 or SEQ ID NO: 4. Examples of such an improved function include increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, decreased aggregation, and increased solubility. Such a mutein is produced by a method. . . having such an improved function. The present invention also includes a mutein having an improved function compared to an unmodified ***Fc*** .epsilon.RI.alpha. protein, wherein the amino acid sequence of the mutein differs in at least one position from the amino acid sequence. . .

SUMM [0016] Also included are muteins that are chemically modified ***Fc*** .epsilon.RI.alpha. proteins. Also included are nucleic acid molecules that encode muteins of the present invention, recombinant molecules and recombinant cells including. . .

SUMM [0017] The present invention also includes a method to improve a function of a ***Fc*** .epsilon.RI.alpha. protein which includes the steps of: (a) analyzing a 3-D model of an extracellular domain of a ***human*** high affinity ***Fc*** .epsilon.RI.alpha. protein substantially representing the atomic coordinates specified in Table 1, Table 5, Table 6, Table 7 or Table 8, to. . .

DRWD [0018] FIG. 1 depicts electron density maps and overall structure of a ***human*** ***Fc*** .epsilon.RI.alpha. model. (A) The 3.0 angstrom experimental electron density map, calculated using the MIRAS phases followed by density modification with the program DM is shown along with a refined model for ***human*** ***Fc*** .epsilon.RI.alpha.. The density is contoured at 1.4.sigma. for residues 147-153. (B) Electron density for carbohydrate moieties linked to N42. The |2Fo- ***Fc*** | electron density map, contoured at 1.sigma., was calculated to 2.4 angstroms using combined MIRAS and model phases (prior to inclusion. . .

DRWD [0019] FIG. 2 depicts a ribbon diagram of a ***human*** ***Fc*** .epsilon.RI.alpha. model showing the positions of the disulfides and the

FG loop in domain 2 (D2) that is implicated in receptor. . .

DRWD [0020] FIG. 3 depicts a topology diagram of the two domains of a ***human*** ***Fc*** .epsilon.RI.alpha. model showing the hydrogen-bonding patterns of the beta sheet structure. The short stretch of parallel beta-sheet in D1 and D2. . .

DRWD [0021] FIG. 4 demonstrates that a ***human*** ***Fc*** .epsilon.RI.alpha. model has a novel tertiary arrangement of tandem Ig domains.

DRWD [0022] FIG. 5 depicts sequence alignments of ***human*** ***FcRs*** . The secondary structure of the two domains is indicated with labeled bars above those residues which form beta-sheet in ***Fc*** .epsilon.RI. Below the sequences, carbohydrate attachment sites found in seventeen different ***FcR*** sequences are indicated with a (+). This analysis is based on the seven ***human*** receptors shown and the non- ***human*** receptors listed in Table 4.

DRWD [0023] FIG. 6 depicts the four surface-exposed tryptophans at the top of the D2 domain of a ***human*** ***Fc*** .epsilon.RI.alpha. model that are implicated in IgE binding.

DRWD [0024] FIG. 7 depicts residues in the D2 FG loop and D1 E strand of a ***human*** ***Fc*** .epsilon.RI.alpha. model that are highly variable in ***human*** ***FcR*** sequences. The residues in the D2-FG loop have been directly implicated in IgE binding. The residues in the D1 E. . .

DRWD [0025] FIG. 8 depicts a juxtaposition of a ***human*** ***Fc*** .epsilon.RI.alpha. model with a model for the intact IgE antibody structure. The insertion of the C.epsilon.2 domains in the IgE molecule are indicated by dotted lines. The ***Fc*** .epsilon.RI.alpha. protein is shown relative to the mast cell membrane near the top of the C.epsilon.3 domains that bind to the. . .

DETD [0026] The present invention includes isolated crystals of the extracellular domains of ***FcRs*** , 3-D models of such crystals and modifications of such models. The present invention also includes compounds that inhibit the ability of ***FcRs*** to bind to antibodies as well as muteins and other modified ***FcRs*** . Also included in the present invention are methods to produce and use such crystals, models, inhibitory compounds, muteins, and other. . .

DETD [0027] The present invention includes an isolated crystal of an extracellular domain of a high affinity ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.RI.alpha.), a 3-D model of such a crystal and a modification of such a model. As used herein, the term "a". . .

DETD [0028] As used herein, an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein is the portion of the ***FceRI*** alpha chain that is exposed to the environment outside the cell and that binds to the ***Fc*** domain of an IgE antibody. Such an extracellular domain can be (a) a complete extracellular domain which is a domain that extends from the first amino acid of a mature ***FceRI*** alpha chain through the last amino acid prior to the start of the transmembrane region or a domain that is. . . a domain includes a D1 and D2 domain, displays a similar affinity for the IgE antibody to which such an ***Fc*** .epsilon.RI.alpha. protein naturally binds, and produces crystals having sufficient quality to enable structure determination, or (b) a fragment of any of the extracellular domains of (a), wherein the fragment retains its ability to bind to the ***Fc*** domain of an antibody. As used herein, the terms binding to an antibody and binding to the ***Fc*** domain (i.e., constant region) of an antibody can be used interchangeably since it is recognized that a ***FcR*** binds to the ***Fc*** domain of an antibody. A ***FcR*** (i.e., a protein that can bind to an antibody), such as a ***Fc*** .epsilon.RI.alpha. protein, can be a full-length ***FcR*** (e.g., a full-length ***FceRI*** alpha chain), or any fragment thereof, wherein the fragment binds to an antibody. Similarly an antibody, or an ***Fc*** domain thereof, can be a full-length antibody, or full-length ***Fc*** domain thereof, or any fragment thereof that binds to a ***FcR*** . Preferably a ***FcR*** binds to an antibody with an affinity (K.sub.A) of at least about 10.sup.8 liters/mole (M.sup.-1), more preferably of at least. . .

DETD . . . in several aspects. For example, this is the first report of an isolated crystal of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein, and in particular of an isolated crystal of sufficient quality that a crystal structure, i.e., a 3-D model, could.

. . and non-obvious and has been attempted by others without success. The inventors tried many approaches before discovering that a preferred ***Fc*** .epsilon.RI.alpha. protein from which to make a useful crystal is a ***Fc*** .epsilon.RI.alpha. protein that consists of amino acids 1 through 176 of the mature ***human*** ***Fc*** .epsilon.RI.alpha. protein. This protein is denoted herein as PhFc.epsilon.RI.alpha..sub.1-176, or the hFc.epsilon.RI.alpha..sub.1-176 protein, and has an amino acid sequence denoted herein. . . sufficient quality to solve its crystal structure using insect-cell produced PhFc.epsilon.RI.alpha..sub.1-176, a number of other proteins were tried, including a ***Fc*** .epsilon.RI.alpha. protein spanning from amino acid 1 through 171 of SEQ ID NO: 2 produced in Pichia pastoris, and ***Fc*** .epsilon.RI.alpha. proteins spanning from amino acid 1 through 172 of SEQ ID NO: 2 produced in Chinese hamster ovary cells, Trichoplusia. . . determination and that, in order to form a crystal of sufficient quality to determine the first 3-D model of a ***Fc*** .epsilon.RI.alpha. protein, at least one additional amino acid was required carboxyl-terminal to that at position 172; the inventors further believe that. . . 174 of SEQ ID NO: 2. It should be noted, however, that having solved the crystal structure of a first ***Fc*** .epsilon.RI.alpha. protein enables the solving of crystal structures of additional ***Fc*** .epsilon.RI.alpha. proteins as well as of additional ***FcRs*** in general. For example, the crystal structures of two additional crystals cited in the Examples can be solved using a. . . the solution of an additional four crystal structures using such information, namely the examples present 3-D models of: (a) a ***human*** ***Fc*** .epsilon.RI.alpha. protein spanning amino acids 1-172 of SEQ ID NO: 2 (i.e., PhFc.epsilon.RI.alpha..sub.1-172, the amino acid sequence of which is represented. . .

DETD . . . to date all of which have only identified mutations in D2 that lead to decreased, or increased, binding between a ***Fc*** .epsilon.RI.alpha. protein and an IgE antibody. As such, a model of the present invention is necessary for proper interpretation and refinement. . . a model for the first time permits the differentiation between amino acids directly or indirectly influencing binding of IgE to ***Fc*** .epsilon.RI.alpha. and demonstrates where amino acids and amino acid segments identified in mutagenesis and swapping studies are positioned on the protein. It is to be noted that the 3-D models of ***Fc*** .epsilon.RI.alpha. crystal structure forms T1, T2, M2 and H1 are quite similar to that of form M1, with the following differences.. . .

DETD [0031] One embodiment of the present invention is an isolated crystal of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein. As used herein, an isolated crystal is a crystal of a protein that has been produced in a laboratory;. . . effect crystallization; such precipitants are known to those skilled in the art. In a preferred embodiment, a crystal of a ***Fc*** .epsilon.RI.alpha. protein is produced in a solution by adding a precipitant such as polyethylene glycol (PEG) or PEG monomethylether. In a. . . 40%, preferably from about 12% to about 32% PEG per volume solution. It is also to be noted that a ***Fc*** .epsilon.RI.alpha. protein used to produce a crystal can be produced by a variety of methods, including purification of a native protein,. . .

DETD . . . present invention are not derivatized. In one embodiment, an isolated crystal of the present invention is a co-crystal of a ***Fc*** .epsilon.RI.alpha. protein bound to a ***Fc*** domain of an IgE antibody. In another embodiment, an isolated crystal of the present invention is a co-crystal of a ***Fc*** .epsilon.RI.alpha. protein and a compound that inhibits the binding of a ***Fc*** .epsilon.RI.alpha. protein to a ***Fc*** domain of an IgE antibody. Additional crystals of the present invention include crystals produced from proteins that are muteins of. . .

DETD [0033] An isolated crystal of the present invention can be the crystal of any suitable extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein. Suitable ***Fc*** .epsilon.RI.alpha. proteins include mammalian ***Fc*** .epsilon.RI.alpha. proteins, with ***human*** , canine, feline, equine, rat and murine ***Fc*** .epsilon.RI.alpha. proteins being preferred, and ***human*** ***Fc*** .epsilon.RI.alpha. proteins being even more preferred. A preferred crystal of the present invention diffracts X-rays to a resolution of

about 4.0. . . amino acid sequence SEQ ID NO: 4, or a sequence essentially equivalent that represents an extracellular domain of another mammalian ***Fc*** .epsilon.RI.alpha. protein. SEQ ID NO: 4 is the amino acid sequence of a protein consisting of the first 172 residues of a mature ***human*** ***Fc*** .epsilon.RI.alpha. protein denoted herein as PhFc.epsilon.RI.alpha..sub.1-172; i.e., SEQ ID NO: 4 spans from amino acid residue 1 through amino acid residue. . .

DETD [0034] The present invention includes a 3-D model of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein that substantially represents the atomic coordinates specified in Table 1, Table 5, Table 6, Table 7 or Table 8.. . . 1, Table 5, Table 6, Table 7 or Table 8. Each such modification represents a protein that binds to a ***Fc*** domain of an antibody. A 3-D model of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein is a representation, or image, that predicts the actual structure of the corresponding protein. As such, a 3-D model. . . relationship between the protein's structure and function at the atomic level and to design muteins (i.e., genetically and/or chemically altered ***FcRs***) having an improved function, such as, but not limited to: increased (i.e., enhanced) stability; increased antibody binding activity, for example,. . . increasing the affinity for an antibody by, for example, increasing the association rate and/or decreasing the dissociation rate between a ***FcR*** and an antibody or by altering substrate specificity (e.g., enhancing the ability of a ***FcR*** of a certain species and class to bind to antibody from another species and/or another antibody class); and/or increased solubility. . . crystal." Furthermore, the model can be subjected to further refinements to more closely correspond to the actual structure of a ***FcR*** . Such a refined model, which is an example of a modification of the present invention, is a better predictor of. . . the model represents. A refinement of a 3-D model of the present invention refers to an improved model of a ***Fc*** .epsilon.RI.alpha. protein that can be obtained in a variety of ways known to those skilled in the art. Refinements can include. . .

DETD [0035] One embodiment of the present invention is a 3-D model of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein that substantially represents the atomic coordinates specified (i.e., listed) in Table 1.

TABLE 1

Atomic coordinates of PhFc.epsilon.RI.alpha..sub.1-176, Form M1

ATOM. . .

DETD [0037] Additional embodiments of the present invention include 3-D models of extracellular domains of ***Fc*** .epsilon.RI.alpha. proteins that substantially represent the atomic coordinates specified in Table 5, Table 6, Table 7 or Table 8, each of. . .

DETD . . . modification, also referred to herein as a model modification, is a model that represents a protein that binds to a ***Fc*** domain of an antibody. A model modification includes, but is not limited to: a refinement of the model that substantially. . . the atomic coordinates specified in Table 1, Table 5, Table 6, Table 7 or Table 8 that binds to a ***Fc*** domain of an antibody; a model based on other ***Fc*** .epsilon.RI.alpha. protein crystals, such as a model based on one or more of the crystals disclosed in the Examples; a model. . . produced using homology modeling techniques to, for example, incorporate all or any part of the amino acid sequence of another ***FcR*** into a 3-D model of the extracellular domain of the model substantially representing the atomic coordinates specified in Table 1,. . . Table 6, Table 7 or Table 8 or incorporate all or any part of the amino acid sequence of a ***Fc*** .epsilon.RI.alpha. protein into a 3-D model of another ***FcR*** ; and a modification representing a ***FcR*** with an altered function, which preferably can be used to design a mutein with an improved function compared to an. . .

DETD . . . model, for example by extracting coordinates from a picture or placing a similar immunoglobulin domain into the 3-D model of ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein form M1, ***Fc*** .epsilon.RI.alpha..sub.1-176 protein form M2, ***Fc*** .epsilon.RI.alpha..sub.1-172 protein form T1, ***Fc*** .epsilon.RI.alpha..sub.1-172 protein form T2, or ***Fc*** .epsilon.RI.alpha..sub.1-172 protein form H1 and deriving a model of the similar domain. Physical 3-D models are tangible and include, but are.

. .

DETD . . . identifies the solvent accessibility of amino acid residues of the corresponding protein. The solvent accessibilities of the amino acids in ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein (form M1) are indicated in Table 2.

TABLE 2

PhFc.epsilon.RI.alpha..sub.1-176, Form M1, residue exposure
>>>> Surface plot for:
>>>> structure file = ***fcr10*** _gen.mtf
>>>> coordinate set = ***fcr10b*** .pdb

resid	resname	access	access-main	access-side
4	LYS	18.7522	5.5920	29.2803
5	PRO	0.5301	0.7105	0.2895
6	LYS	14.4465	0.5227	25.5856
7	VAL	1.6658	2.9151	0.0000

8. . .

DETD [0041] The solvent accessibilities of the amino acids in ***human*** ***Fc*** .epsilon.RI.alpha. protein forms T1, T2, M2 and M1 are indicated in Tables 9, 10, 11, and 12 respectively, each of which. . .

DETD . . . acids that are on the external surface of the protein and, as such, may be involved in binding of a ***FcR*** to an antibody and as such be useful in designing proteins with an enhanced binding activity or in identifying compounds that inhibit such binding. In addition, solvent accessible residues can represent targets for modification to produce a ***FcR*** with improved function. Such analysis also identifies residues in the interior, or core, of the protein. Such residues can also. . . crystals and predict the location of the IgE binding domain, including those amino acids that actually form contacts with a ***Fc*** domain of an IgE antibody, such as those in the binding face of the ***Fc*** .epsilon.RI.alpha. protein. A model can also identify the amino acids in the interface between domain 1 and domain 2 (i.e., the. . .

DETD [0043] One embodiment of the present invention is a model that represents a protein that binds to a ***Fc*** domain of an IgE antibody with an affinity that is at least equivalent to the affinity of the extracellular domain of ***human*** ***Fc*** .epsilon.RI.alpha. for any one of the following IgE antibodies: a ***human*** IgE antibody, a canine IgE antibody, a feline IgE antibody, an equine IgE antibody, a rat IgE antibody, and a murine IgE antibody. Such a model can represent an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, a canine ***Fc*** .epsilon.RI.alpha. protein, a feline ***Fc*** .epsilon.RI.alpha. protein, an equine ***Fc*** .epsilon.RI.alpha. protein, a murine ***Fc*** .epsilon.RI.alpha. protein, and a rat ***Fc*** .epsilon.RI.alpha. protein. Such a model can also represent a protein with altered substrate specificity, preferably designed based on a model of the present invention. WO 98/23964, ibid., reports the ability of an isolated ***human*** ***Fc*** .epsilon.RI.alpha. protein to bind to canine, feline and equine IgE antibodies. Models of the present invention can be used to design a ***FcR*** with increased affinity for an antibody of a species other than self, such as, but not limited to, a ***human*** ***Fc*** .epsilon.RI.alpha. with increased affinity for a canine, feline or equine IgE antibody.

DETD [0044] The present invention includes a model that represents a ***FcR*** that binds to an antibody of its respective class (i.e., IgE, IgG, IgM, IgA or IgD antibody class). Also included is a model that represents a ***FcR*** designed to bind to an antibody of a class other than the class to which the protein naturally binds. Such. . . invention can be produced, for example, by incorporating all or any part of the amino acid sequence of the other ***FcR*** into a 3-D model of the extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein. Such an embodiment includes any model that specifically incorporates any Ig domains that are placed in an orientation (packing interfaces and bend angles) that is based on the structure of the ***Fc*** .epsilon.RI.alpha.. A preferred model of the present invention represents a ***FcR*** that binds to an IgE antibody or to an IgG antibody. In one embodiment, a model of the present invention is a 3-D model of an extracellular antibody binding

domain of a ***FcR*** other than ***human*** ***Fc*** .epsilon.RI.alpha., such as of a ***FcR*** that binds to an IgG antibody. Such proteins and models thereof can be designed by homology modeling by, for example, altering the substrate specificity of a ***Fc*** .epsilon.RI.alpha. protein such that the altered protein binds an IgG antibody.

DETD . . . or Table 8, and more particularly atomic coordinates specified in Table 1. In this embodiment, such a model represents a ***FcR*** that binds to an antibody. The backbone atoms are those atoms that form the backbone, or 3-D folding pattern, of. . . more preferably at least about 60% and even more preferably at least about 80% amino acid sequence homology, with a ***human*** ***Fc*** .epsilon.RI.alpha. protein, as determined using the program ALIGN with default parameters, optimal global alignment of two sequences with no short-cuts. It is to be noted that, using the same program and parameters, the extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein (i.e., soluble ***human*** ***Fc*** .epsilon.RI.alpha. protein) shares about 48% identity with feline and rat soluble ***Fc*** .epsilon.RI.alpha. proteins, about 49% with a murine soluble ***Fc*** .epsilon.RI.alpha. protein, about 50% identity with a canine soluble ***Fc*** .epsilon.RI.alpha. protein, and about 60% identity with an equine soluble ***Fc*** .epsilon.RI.alpha. protein. A preferred model of the present invention represents an IgE binding domain, i.e., a region that binds to an. . .

DETD [0046] One embodiment of the present invention is a 3-D model of a ***human*** ***Fc*** .epsilon.RI.alpha. protein produced by a method that includes the steps of: (a) crystallizing an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, such as, but not limited to a protein having amino acid sequence SEQ ID NO: 2 or SEQ ID. . . can be produced using a variety of techniques well known to those skilled in the art. As disclosed herein, a ***human*** ***Fc*** .epsilon.RI.alpha. protein to be crystallized is preferably produced in recombinant insect cells transformed with a gene encoding an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, such as a baculovirus genetically engineered to produce the protein. The purity of the ***Fc*** .epsilon.RI.alpha. protein must be sufficient to permit the production of crystals that can be analyzed by X-ray crystallography to a resolution. . .

DETD [0047] As disclosed herein, a preferred method to crystallize a ***Fc*** .epsilon.RI.alpha. protein is by vapor distillation. Particularly preferred methods are disclosed in the Examples. It should be appreciated that the present. . .

DETD . . . Crystallography, Academic Press, London, 1976. However, as discussed herein, elucidation of the crystal structure of the extracellular domain of the ***human*** ***Fc*** .epsilon.RI.alpha. was difficult. In one embodiment, crystal structure determination includes obtaining high-resolution data using synchrotron radiation. Such data can be collected,. . .

DETD [0049] One embodiment of the present invention is a method to produce a 3-D model of a ***Fc*** .epsilon.RI.alpha. protein that includes positioning amino acid representations (i.e., representing amino acids) of the protein at substantially the coordinates listed in. . .

DETD . . . frozen crystals; introduction of solvent molecules to the structure; clarification of secondary structure; and analyses of crystallized complexes between a ***FcR*** and an antibody or inhibitory compound. An additional model refinement method includes analyzing a 3-D model to predict amino acid. . .

DETD [0051] Another embodiment of the present invention is a modified 3-D model that represents a ***FcR*** other than a ***human*** ***Fc*** .epsilon.RI.alpha. protein represented by the 3-D model the coordinates of which are listed in Table 1, Table 5, Table 6, Table. . . preferably by incorporating (e.g., grafting, overlaying or replacing) all or any portion of the amino acid sequence of the other ***FcR*** into the 3-D model of the ***human*** ***Fc*** .epsilon.RI.alpha. protein to produce the modified model which comprises the other ***FcR*** . General techniques for homology modeling, also referred to as molecular replacement, have been disclosed in, for example, Greer, 1990, Proteins:. . . Function, and Genetics 8, 30-43; and Lattman, 1985, Methods Enzymol 115, 55-77. However, such technology has not been applied to ***FcRs*** since, until the present invention, no 3-D model of any ***FcR*** was available. Thus, the present invention

now allows the solving of the structures of a number of other natural and mutated forms of ***FcRs*** or any other protein with significant amino acid homology, especially to the functional Ig domains of the ***human*** ***Fc*** .epsilon.RI.alpha. protein.

DETD [0052] In one embodiment, a model of a ***FcR*** , such as, but not limited to a ***Fc*** .epsilon.RI.alpha. protein, is produced by extracting the 3-D coordinates from a published figure or building a 3-D model with atoms from other Ig domains wherein the Ig domains are oriented as predicted for a ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein or a ***Fc*** .epsilon.RI.alpha..sub.1-172 protein. For example, a model of the present invention can be produced by orienting two known Ig domains into a bent confirmation similar to that of the two domains of the ***human*** ***Fc*** .epsilon.RI.alpha. protein. Such a model is referred to as a model in which domain 1 and domain 2 are oriented in. . .

DETD [0053] Suitable ***FcRs*** for which a 3-D model can be determined using homology modeling include any mammalian ***FcR*** , such as a protein that binds to IgE, IgG, IgM, IgA or IgD antibodies. Preferred is a protein that binds to an IgE antibody or an IgG antibody. Preferred ***FcRs*** that bind to IgE include ***human*** , canine, feline, equine, murine and rat ***Fc*** .epsilon.RI.alpha. proteins. The present invention also includes the use of other Ig domains to produce models of the present invention.

DETD [0054] One embodiment of the present invention is a 3-D model of a ***FcR*** having an improved function compared to an unmodified protein as well as a method to produce such a modified model.. . . be produced by replacing at least one amino acid based on information derived from analyzing the 3-D model of a ***Fc*** .epsilon.RI.alpha. protein, such as the model of a ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein or a ***Fc*** .epsilon.RI.alpha..sub.1-172 protein, such that the replacement leads to a protein with an improved function. As used herein, a replacement refers to. . . or the entire protein to, for example, introduce non-natural amino acids or other chemical compounds into the structure of a ***FcR*** . For example, based on a structure of the present invention, one can design synthetic peptides or larger proteins that could. . .

DETD . . . invention includes use of a 3-D model of the present invention to identify a compound that inhibits binding between a ***FcR*** and an antibody. The advantages of using a 3-D model to identify inhibitory compounds are multi-fold in that the model depicts the site at which a ***Fc*** domain of an antibody binds to its ***FcR*** , i.e., the antibody-binding domain, also referred to as the antibody binding site. As such, a large number of potential inhibitory. . . to identify inhibitory compounds include, but are not limited to, designing inhibitory compounds based on the 3-D model of a ***FcR*** , probing such a 3-D model with compounds that are potential inhibitors in order to identify those compounds that are actually inhibitory of the binding of an antibody to its ***FcR*** , screening a compound data base using such a 3-D model to identify compounds that inhibit such binding, and combinations thereof.. . .

DETD [0056] An inhibitory compound can be any natural or synthetic compound that inhibits the binding of an antibody to a ***FcR*** . Examples include, but are not limited to, inorganic compounds, oligonucleotides, proteins, peptides, antibodies, antibody fragments, mimetics of peptides or antibodies. . . interact at the binding site or allosterically. An inhibitory compound should be capable of physically and structurally associating with a ***FcR*** and/or an antibody such that the compound can inhibit binding between the two entitites. As such, an inhibitory compound is. . . be identified in one or multiple steps. For example, a compound initially identified that inhibits binding between an antibody and ***FcR*** to some extent can be used as a lead to design, probe or screen for a compound with improved characteristics,. . .

DETD . . . the present invention is a method to identify a compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein. Such a method includes the step of using a 3-D model substantially representing the atomic coordinates specified in Table. . . binding domain or the receptor binding domain of the IgE antibody as well as compounds that interact indirectly with an

Fc .epsilon.RI.alpha. protein, such as compounds that interact with the D1D2 interface, with the cleft between D1 and D2, with a region. . . a combination of 3-D model and mutagenesis analysis to indirectly affect antibody binding, a region suggested by homology with other ***Fc*** .epsilon.RI.alpha. proteins of other species, a region suggested by homology with other ***FcRs*** . In a preferred embodiment, an inhibitory compound interacts with at least one of the following regions of a model representing a ***Fc*** .epsilon.RI.alpha. protein: a A'B loop of D1, a EF loop of D1, a BC loop of D2, a C strand of. . .

DETD [0058] One embodiment of a method to identify a compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein includes the steps of: (a) generating a model substantially representing the atomic coordinates listed in Table 1, Table 5,. . . wherein such an interaction indicates that the compound is capable of inhibiting the binding of an IgE antibody to a ***Fc*** .epsilon.RI.alpha. protein. In a preferred embodiment, step (a) includes the step of identifying one or more amino acid(s) in the IgE binding domain of the model that interact directly with the ***Fc*** domain of an IgE antibody when the ***Fc*** domain binds to the IgE binding domain. Preferably a compound to be tested will interact directly with one or more. . .

DETD . . . of the present invention to expand the use of models of the present invention to produce models of any suitable ***FcRs*** (i.e., model modifications) and to identify compounds that inhibit the binding of antibodies to such ***FcRs*** .

DETD . . . invention also includes use of a 3-D model of the present invention to rationally design and construct modified forms of ***FcRs*** that have one or more improved functions, such as, but not limited to, increased activity, increased stability and increased solubility compared to an unmodified ***FcR*** . Muteins of the present invention include full-length proteins as well as fragments (i.e., truncated versions) of such proteins.

DETD [0061] One embodiment of the present invention is a ***FcR*** that comprises a mutein that binds to a ***Fc*** domain of an antibody. Such a mutein has an improved function compared to a protein comprising SEQ ID NO: 2. . . NO: 4. Examples of such an improved function include, but are not limited to, increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, and increased solubility. Such a mutein can be produced by a method that. . . disrupt the 3-D structure of the protein; i.e., the modified protein, or mutein, is still capable of binding to the ***Fc*** domain of an antibody. A preferred mutein is a ***FcR*** that binds to a ***Fc*** domain of an IgE antibody, although the invention also covers muteins binding to other classes of antibodies.

DETD . . . concentrations, to oxidation and/or reduction, to deamidation, to other forms of chemical degradation and to proteolytic degradation compared to unmodified ***FcR*** . Increased stability can also refer to the ability of a mutein of the present invention to be stable for a. . . thiocyanate, etc. A preferred mutein of the present invention has a melting temperature substantially higher than that of an unmodified ***FcR*** . Preferably the melting temperature of a mutein is at least about 1.degree. C. higher, and more preferably at least about. . .

DETD [0063] Another embodiment of the present invention is a mutein that exhibits increased affinity for a ***Fc*** domain of an antibody compared to its unmodified counterpart. As used herein, a mutein having increased affinity is a ***FcR*** that exhibits a higher affinity constant (K.sub.A) or lower dissociation constant (K.sub.D) than its unmodified counterpart. Such a higher affinity constant can be achieved by increasing the association rate (k.sub.a) between the mutein and the ***Fc*** domain and/or decreasing the dissociation rate (k.sub.d) between the mutein and the ***Fc*** domain. A preferred mutein of the present invention has a K.sub.A for a ***Fc*** domain of at least about 3.times.10.sup.9 liters/mole (M.sup.-1), which is equivalent to a K.sub.D of less than or equal to about 3.3.times.10.sup.-10 moles/liter (M). More preferred is a mutein having a K.sub.A for a ***Fc*** domain of at least about 2.times.10.sup.10 M.sup.-1, and even more preferably of at least about 1.times.10.sup.11 M.sup.-1. Also preferred is a mutein having a k.sub.a for a ***Fc*** domain of at least about 1.times.10.sup.5 liters/mole-second as well as a mutein having a k.sub.d for a ***Fc*** domain of less than or equal to

3.times.10.sup.-5/second. More preferred is a mutein having a k.sub.a for a ***Fc*** domain of at least about 3.times.10.sup.5 liters/mole-second, and even more preferably of 1.times.10.sup.6 liters/mole-second. Also preferred are muteins having a k.sub.d for a ***Fc*** domain of less than or equal to 1.times.10.sup.-5/second or even more preferably less than or equal to 3.times.10.sup.-4/second. A preferred ***Fc*** domain is that of an IgE antibody. Methods to measure such binding constants is well known to those skilled in the art; see, for example, Cook et al., 1997, ibid., which reports the following values for the binding of ***human*** ***Fc*** .epsilon.RI.alpha. protein to ***human*** IgE: k.sub.a1 of 3.5 (.+-.0.9).times.10.sup.5 M.sup.-1s.sup.-1; k.sub.a2 of 8.6 (.+-.3.5).times.10.sup.4 M.sup.-1s.sup.-1; k.sub.d1 of 1.2 (.+-.0.1).times.10.sup.-2s.sup.-1; k.sub.d2 of 3.2 (.+-.0.8).times.10.sup.-5s.sup.-1; K.sub.A1. . .

DETD . . . to its unmodified counterpart. A mutein exhibiting altered substrate specificity is a mutein that binds with increased affinity to a ***Fc*** domain of an antibody class or antibody species of a different type than that normally bound by its unmodified counterpart. In one embodiment, a mutein of a ***human*** ***Fc*** .epsilon.RI.alpha. protein with altered substrate specificity is a ***FcR*** that binds with increased affinity to a IgE antibody of another mammal, such as, but not limited to, a canine, feline, equine, murine, or rat IgE antibody. In another embodiment, a mutein of a ***human*** ***Fc*** .epsilon.RI.alpha. protein with altered substrate specificity is a ***FcR*** that binds with increased affinity to an antibody of another class, such as IgG, IgM, IgA, or IgD, with IgG. . .

DETD [0066] As disclosed herein, the 3-D model representing a ***Fc*** .epsilon.RI.alpha. protein is advantageous in determining strategies for producing muteins having an improved function, e.g., for identifying targets to modify in order to obtain muteins having improved functions. Examples of targets are as follows. A key feature of the ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein or the ***Fc*** .epsilon.RI.alpha..sub.1-172 protein is the crystal contacts in five space groups, a subset of which are predicted to interact directly with a ***Fc*** domain of an IgE antibody. Such contacts are included in the IgE binding domain which is unique for ***human*** ***Fc*** .epsilon.RI.alpha. in that the domain includes a tryptophan-containing hydrophobic ridge positioned on the top face of the crystal structure (i.e., amino. . . or SEQ ID NO: 4. Yet another striking feature is the finding that the amino and carboxyl termini of the ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein are only 10 angstroms apart.

DETD . . . present invention to produce the mutein having the improved function. Knowledge of the structure of the extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein crystal, for example, permits the rational design and construction of modified forms of the protein by permitting the prediction. . . bridges, hydrophobic interactions and hydrogen bonds unless the goal is to purposefully change such interactions. The 3-D structure of the ***human*** ***Fc*** .epsilon.RI.alpha. protein suggests that large deletions may not be desirable, particularly due to the relation between the various domains of the. . .

DETD [0069] In accordance with the present invention, the 3-D model of the ***human*** ***Fc*** .epsilon.RI.alpha. protein has been analyzed, using techniques known to those skilled in the art, to determine the accessibility of the amino. . .

DETD . . . a method to produce a mutein includes the steps of (a) comparing a key region of a model of a ***human*** ***Fc*** .epsilon.RI.alpha. protein with the amino acid sequence of a ***FcR*** having an improved function compared to the unmodified ***Fc*** .epsilon.RI.alpha. protein in order to identify at least one amino acid segment of the ***FcR*** with the improved function that if incorporated into the ***Fc*** .epsilon.RI.alpha. protein represented by the model would give the ***Fc*** .epsilon.RI.alpha. protein the improved function; and (b) incorporating the segment into the ***Fc*** .epsilon.RI.alpha. protein, thereby providing a mutein with the improved function. In another embodiment, a method to produce a protein includes the steps of: (a) using a model representing a ***human*** ***Fc*** .epsilon.RI.alpha. protein to identify a 3-D arrangement of

residues that can be randomized by mutagenesis to allow the construction of a. . . a method that includes the steps of: (a) effecting random mutagenesis of nucleic acid molecules encoding a target of a ***Fc*** .epsilon.RI.alpha. protein as identified by analyzing a model of that protein, such as an IgE binding domain; (b) cloning such mutagenized. . .

DETD . . . at least one amino acid in at least one non-constrained loop of domain 1 in an area proximal to the ***FceRI*** gamma chain putative binding site; (b) joining an amino-terminal amino acid residue to a carboxyl-terminal amino acid residue of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein; (c) replacing at least one amino acid site with an amino acid suitable for derivatization; (d) replacing at least. . .

DETD . . . or chemical synthetic methods of a nucleic acid molecule encoding the desired protein, such as, but not limited to a ***human*** ***Fc*** .epsilon.RI.alpha. protein, followed by expression of the mutated gene in a suitable expression system, preferably an insect, mammalian, bacterial, yeast, insect,. . .

DETD . . . present invention is a mutein in which at least one amino acid in at least one non-constrained loop of a ***Fc*** .epsilon.RI.alpha. protein is replaced in order to improve a function of the protein. Finding that the ***human*** ***Fc*** .epsilon.RI.alpha. protein had such loops was surprising, and it is believed, without being bound by theory, that a mutein in which. . . 31-35 and 70-74 of SEQ ID NO: 2 or SEQ ID NO: 4), preferably in an area proximal to the ***FceRI*** gamma chain putative binding site, i.e., the site on the ***Fc*** .epsilon.RI.alpha. protein to which the gamma chain of the high affinity ***Fc*** ***epsilon*** ***receptor*** is thought to bind. In a preferred embodiment, one or more amino acids is replaced to make loops shorter, but. . .

DETD [0074] Another embodiment of the present invention is a mutein of the extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein in which an N-terminal (amino-terminal) amino acid residue is joined, preferably covalently, to a C-terminal (carboxyl-terminal) amino acid residue in order to improve a function of the protein. Finding that the N-termini and C-termini of the ***human*** ***Fc*** .epsilon.RI.alpha. protein were only 10 angstroms apart was quite surprising. Without being bound by theory, it is believed that such a. . .

DETD . . . site, is replaced with a charged or polar residue to increase solubility or create more stable muteins. Glycosylation sites in ***human*** ***Fc*** .epsilon.RI.alpha. protein include amino acids 21, 42, 50 74, 135, 140, and 166 of SEQ ID NO: 2 or SEQ ID. . .

DETD . . . order to improve the function of the mutein, at least by increasing stability. Cysteine pairs can be substituted into a ***Fc*** .epsilon.RI.alpha. protein at any two residue positions identified with available programs and algorithms that would allow the formation of an undistorted. . .

DETD . . . N-linked glycosylation sites are added to or removed from the protein, preferably by substitution with an appropriate amino acid. A ***Fc*** .epsilon.RI.alpha. protein with additional N-linked glycosylation sites is more soluble. The ability to design a ***Fc*** .epsilon.RI.alpha. protein having fewer, or no, N-linked glycosylation sites is also valuable as production of such a protein from production run to production run is likely to be more uniform. One embodiment is a ***Fc*** .epsilon.RI.alpha. mutein with no N-linked glycosylation sites that is stable, active, and soluble. Such a protein has an advantage of being. . .

DETD [0087] Another embodiment of the present invention to enhance stability is the addition of polyethylene glycol (PEG) groups to a ***FcR*** protein, i.e., to produce a "pegylated" ***FcR*** protein. In one embodiment, the PEG group(s) can substitute for carbohydrate group(s) due to removal of one or more N-glycosylation. . .

DETD [0088] Another embodiment of the present invention is a mutein that comprises a ***FcR*** having a substance, such as a ligand or ***detectable*** ***marker*** , attached to an amino acid of the protein such that the substance does not substantially interfere with the antibody binding. . . its function, such as binding to a second member of a ligand pair or enabling detection of the protein. The ***FcR*** to which a substance is attached can be either an unmodified protein or a mutein of the present invention. Suitable. . . nature of

the amino acid prior to any modification required for attachment. Examples of suitable substances to attach to a ***FcR*** include any compound capable of binding to or reacting with another substance, such as those described for attachment to a. . .

DETD . . . to expand the use of models of the present invention to produce models of and make modifications to any suitable ***FcRs*** or other Ig domain-containing proteins to produce muteins having a desired function.

DETD [0092] The present invention includes an isolated ***Fc*** .epsilon.RI.alpha. protein that consists of SEQ ID NO: 2, i.e., PhFc.epsilon.RI.alpha..sub.1-176. Also included in the present invention is a protein consisting of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein that is structurally homologous to an isolated ***Fc*** .epsilon.RI.alpha. protein consisting of SEQ ID NO: 2. As used herein, a protein that is structurally homologous to PhFc.epsilon.RI.alpha..sub.1-176 is a. . . short-cuts, (c) displays a substantially equivalent affinity for an IgE antibody as does a complete extracellular domain of the corresponding ***Fc*** .epsilon.RI.alpha. protein, and (d) produces crystals having sufficient quality to enable structure determination. Examples of such proteins include a ***human*** ***Fc*** .epsilon.RI.alpha. protein having SEQ ID NO: 4, i.e., PhFc.epsilon.RI.alpha..sub.1-172 and a ***human*** ***Fc*** .epsilon.RI.alpha. protein having an amino acid sequence that spans from amino acid 3 through amino acid 174 of SEQ ID NO:. . . i.e., PhFc.epsilon.RI.alpha..sub.3-174. It is to be noted that these examples are provided to clarify the definition of a structurally homologous ***Fc*** .epsilon.RI.alpha. protein and are not intended to limit the scope of such proteins. That is, a ***Fc*** .epsilon.RI.alpha. protein that is structurally homologous to PhFc.epsilon.RI.alpha..sub.1-176 is any mammalian ***Fc*** .epsilon.RI.alpha. protein having the listed characteristics. Preferred are ***human*** , canine, feline, equine, murine and rat proteins that are structurally homologous to PhFc.epsilon.RI.alpha..sub.1-176. Also included herein are nucleic acid molecules. . .

DETD [0093] The present invention also includes a ***Fc*** .epsilon.RI.alpha. protein consisting of SEQ ID NO: 4 except that the isoleucine at position 170 has been replaced by a cysteine. Also included in the present invention is a protein consisting of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein that is structurally homologous to an isolated ***Fc*** .epsilon.RI.alpha. protein consisting of SEQ ID NO: 4 except that the isoleucine at position 170 has been replaced by a cysteine.

DETD . . . nucleic acid molecules encoding proteins of the present invention, including, but not limited to, proteins comprising unmodified extracellular domains of ***FcRs*** , novel structures within such proteins, and muteins. As used herein, an isolated nucleic acid molecule encoding a protein is a. . .

DETD [0104] The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a ***FcR*** of the present invention (i.e., anti- ***FcR*** antibodies). As used herein, the term "selectively binds to" ***FcR*** refers to the ability of antibodies of the present invention to preferentially bind to specified proteins of the present invention.. . .

DETD [0106] A ***FcR*** of the present invention can include chimeric molecules comprising at least a portion of a ***FcR*** that binds to an antibody and a second molecule that enables the chimeric molecule to be bound to a substrate. . . a manner that the antibody receptor portion binds to the antibody in at least as effective a manner as a ***FcR*** that is not bound to a substrate. An example of a suitable second molecule includes a portion of an immunoglobulin. . .

DETD . . . entireties. It is to be noted that although the compositions and methods disclosed in WO 98/27208, ibid., relate to feline ***Fc*** .epsilon.RI.alpha. proteins, they are also applicable to therapeutic compositions of the present invention. Therapeutic compositions of the present invention are advantageous. . .

DETD . . . entireties. It is to be noted that although the reagents and methods disclosed in WO 98/27208, ibid., relate to feline ***Fc*** .epsilon.RI.alpha. proteins, they are also applicable to diagnostic reagents, kits and detection methods of the present invention. Muteins of the present. . .

DETD [0111] This Example describes the production of a ***Fc***

.epsilon.RI.alpha. nucleic acid molecule, a recombinant molecule, a recombinant cell, a recombinant virus, and a ***Fc*** .epsilon.RI.alpha. protein of the present invention.

DETD [0112] A number of ***human*** ***Fc*** .epsilon.RI.alpha. proteins of variable lengths (i.e., 171, 172, and 176 amino acids) were produced in a variety of cell lines (i.e.,. . . cells). Due to a number of factors, however, including protein length, solubility, and extent and variability of glycosylation, only one ***Fc*** .epsilon.RI.alpha. protein was useful in producing a crystal of sufficient quality for the first determination of a model of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein. The production of this protein is disclosed below.

DETD [0113] A nucleic acid molecule comprising the first 176 amino acids of the mature form of the ***human*** ***Fc*** .epsilon.RI.alpha. protein, nucleic acid molecule and protein designated herein as nhFc.epsilon.RI.alpha..sub.1-528 and PhFc.epsilon.RI.alpha..sub.1-176, respectively, was produced as follows. An EcoRI-HindIII fragment from plasmid EdpC20 (Blank et al., ibid.) containing the ***human*** ***Fc*** .epsilon.RI.alpha. signal sequence and residues 1-172 of the mature ***human*** ***Fc*** .epsilon.RI.alpha. protein was ligated to two oligonucleotides coding for residues 172-176 of the mature protein and two stop codons. The two. . .

DETD [0118] This Example describes the production of a ***Fc*** .epsilon.RI.alpha. protein crystal of the present invention.

DETD [0120] This Example describes the production of additional ***Fc*** .epsilon.RI.alpha. protein crystals of the present invention.

DETD [0121] Nucleic acid molecule nhFc.epsilon.RI.alpha..sub.1-516 , encoding the first 172 amino acids of the ***human*** ***Fc*** .epsilon.RI.alpha. protein was expressed in T. ni Hi-5 cells to produce PhFc.epsilon.RI.alpha..sub.1-172 in a manner similar to that described for the. . .

DETD . . . phase target (MLHL). Brunger et al., ibid., is incorporated by reference herein in its entirety. Specifically, the structure of the ***Fc*** .epsilon.RI.alpha. protein PhFc.epsilon.RI.alpha..sub.1-176 was determined by multiple isomorphous replacement using gold and platinum heavy atom derivatives with the anomalous signal from. . .

DETD [0127] This Example describes the structure of a ***Fc*** .epsilon.RI.alpha. protein predicted by a three-dimensional model of the present invention.

DETD [0129] The model of extracellular domain of the ***human*** ***Fc*** .epsilon.RI.alpha. protein, also referred to herein as the hFc.epsilon.RI.alpha. model or hFc.epsilon.RI.alpha. structure, indicates that PhFc.epsilon.RI.alpha..sub.1-176 is composed of two immunoglobulin. . . domain arrangement generates a flat surface at the top of the receptor that has been implicated in binding to the ***Fc*** domain of an IgE antibody. The domains are small compared to canonical variable and constant Ig domains and the shorter. . .

DETD . . . surfaces between domains. A subset of D1 and D2 representative structures of are shown in FIG. 4, including those from ***human*** ***Fc*** .epsilon.RI.alpha. (designated as IgE- ***FcR***), the natural killer cell inhibitory receptor, (KIR, Fan et al., 1997, Nature 389, 96-100), the ***human*** growth hormone receptor (HGHBP, de Vos et al., 1992, Science 255, 306-312), the interleukin-1 receptor, (IL1R, Vigers et al., 1997,. . . two Ig domains being compared. The figures on top show side views and the figures below show top views. The ***Fc*** .epsilon.RI.alpha. and hemolin structures have the most acute angles relating two sequential Ig domains. The top view of these domains shows that the orientation of the hemolin and ***Fc*** .epsilon.RI.alpha. domains are more closely related, but between this selected subset of proteins there is significant variability in the relative orientations. . .

DETD [0133] The bent shape of the ***Fc*** .epsilon.RI.alpha. structure produces a large interface between the D1D2 domains that buries 1280 .ANG..sup.2 of accessible surface area of 28 D1. . . the interface (87-93, 95, 104, 106, 108, 110-111, 161, 163-165). Of these 28 residues, 8 are completely conserved in all ***human*** ***FcgR*** and ***Fc*** .epsilon.RI.alpha. sequences (corresponding to residues 13, 87, 88, 90, 91, 106, 108, 110 of SEQ ID NO: 2); see FIG. 5. These conserved residues form a significant fraction to one side of the buried interface, suggesting that related ***FcRs*** would have a similar acute packing of the D1D2 domains as observed in the ***Fc***

.epsilon.RI.alpha. model.

DETD [0134] However, 20 residues that form the D1D2 interface in the ***Fc*** .epsilon.RI.alpha. model differ in other ***FcRs*** and these differences could alter the relative orientations of the two domains. For example, the conserved tryptophan at position 110 packs against a phenylalanine at position 17 of ***Fc*** .epsilon.RI.alpha.. In related ***FcRs*** , this phenylalanine is changed to a leucine, which may lead to slight alterations in the packing of the two domains. Another central residue in the ***Fc*** .epsilon.RI.alpha. D1D2 interaction is residue R15, which forms a hydrogen bond with the carbonyl of L90 and is packed against L89, F84, and L165. In related ***human*** ***FcRs*** , arginine 15 is changed to serine or asparagine, which corresponds to a significant volume and charge change at the center of the D1D2 interaction. Since the interactions of the ***FcR*** with antibody are near the D1D2 interface, alterations in residues at the interface might influence receptor specificity. Other residues that are variable amongst the ***FcR*** sequences in the region of the D1D2 could also perturb the D1D2 interactions.

DETD . . . the extracellular regions of the beta or gamma subunits of the receptor. It has been suggested that interactions between the ***FcgRI*** and ***FcgRIIIA*** alpha and gamma subunits increase the binding affinity of the receptor for IgG (Miller et al., 1996, J. Exp. Med. 183, 2227-2233). Although the extracellular regions of the ***human*** ***FceR*** gamma chain are short (about 5 to 7 amino acids), these regions could potentially interact with the D1D2 cleft and thereby affect the affinity of the receptor for antibody. In addition, recent binding studies with recombinant, soluble ***Fc*** .epsilon.RI.alpha. and IgE have demonstrated a 10-fold lower affinity than had previously been determined in cell-binding assays (Cook et al., 1997,. . .

DETD [0137] The ***human*** ***Fc*** .epsilon.RI.alpha. protein PhFc.epsilon.RI.alpha..sub.1-176 is the most highly glycosylated protein structure solved by X-ray crystallography to date, having seven N-linked glycosylation sites in 176 amino-acid residues. The intact ***Fc*** .epsilon.RI.alpha. on mast cells is approximately 40% carbohydrate by weight (Kanellopoulos, et al., 1980, E. J. Biol. Chem. 255, 9060-9066); LaCroix, et al., 1993, ibid.), with a heterogeneous molecular weight on SDS-PAGE gels of about 50 kilodaltons (kD). ***Human*** ***Fc*** .epsilon.RI.alpha. expressed in insect cells has a molecular weight of about 34 kD as observed using SDS-PAGE, but, based on typical. . . (Letourneur et al., 1995, ibid.; Robertson, 1993, ibid.; Scarselli et al., 1993, ibid.), mutation of these sites or treatment of ***FceRI*** -expressing cells with tunicamycin leads to the aggregation of the receptor during biosynthesis.

DETD . . . 42 and 166, three sugar moieties were built. The carbohydrate at position 42 extends up towards the top of the ***Fc*** .epsilon.RI.alpha. structure, covering residues F60, S63 and V83. The carbohydrate attached to position 166 projects away from the protein surface, potentially. . .

DETD [0139] Many of the related ***FcR*** proteins are also highly glycosylated proteins and the glycosylation sites vary between receptors. Rat and mouse ***Fc*** .epsilon.RI.alpha. proteins each have six potential N-linked glycosylation sites, of which two sites and one site, respectively are shared in common with the ***human*** ***Fc*** .epsilon.RI.alpha. protein. Comparison of seventeen ***human*** and animal ***FcR*** sequences identifies twenty-five different potential N-linked carbohydrate attachment sites in related ***FcRs*** . The twenty-five sites are distributed evenly between D1 and D2, with fourteen sites in D1 and eleven sites in D2. Five of these sites are relatively well conserved sites in all ***FcRs*** (found in >9/17 sequences analyzed) and they correspond to residues 35, 42, 61, 135, and 142 of SEQ ID NO: 2. These sites cover a significant fraction of the ***Fc*** .epsilon.RI.alpha. surface on both major faces of D1 and D2, placing limitations on the surface available for interactions with antibody.

DETD [0140] It is not known why ***FcRs*** are so heavily glycosylated. Many potential roles for carbohydrate sites on proteins have been suggested, including specific roles in determining. . . proteins (Huber et al., 1976, Nature 264, 415-420; Vaughn et al., 1998, Structure 6, 63-73). In the case of the ***human*** ***FcRs*** , the number

of potential N-linked glycosylation sites correlates to some degree with the affinity of the ***FcR*** for immunoglobulin. Table 4 shows the number of glycosylation sites in the domains corresponding to the extracellular domain of the ***human*** ***Fc*** .epsilon.RI.alpha. protein along with the total number of glycosylation sites in parentheses. Affinity data are taken from Ravetch et al., 1998,. . . al., 1991, Annu. Rev. Immunol. 9, 457-492.

TABLE 4

Comparison of the number of predicted glycosylation sites and the affinity of different ***FcRs*** for antibody.

FcR	# CHO sites (total)	Affinity
Human		
Fc .epsilon.RI	7	high (10.sup.-9-10.sup.-10 M)
Fc .gamma.RIA (CD64)	5 (7)	high (3 domains, 10.sup.-8-10.sup.-9 M)
Fc .gamma.RIB (CD64)	5 (7)	high (3 domains, 10.sup.-8-10.sup.-9 M)
Fc .gamma.RIIA (CD32)	2 (3)	low (10.sup.-6 M)
Fc .gamma.RIIB (CD32)	3	low (10.sup.-6 M)
Fc .gamma.RIIC (CD32)	3 (4)	low (10.sup.-6 M)
Fc .gamma.RIIIA (CD16)	5 (6 in variant)	low (10.sup.-6 M)
Mouse		
Fc .epsilon.RI	6	high (10.sup.-9-10.sup.-10 M)
Fc .gamma.RI	4(5)	high (3 domains, 10.sup.-7-10.sup.-8 M)
Fc .gamma.RIIb	4(5)	low (10.sup.-6 M)
Fc .gamma.RIIfa	4	low (10.sup.-6 M)
Rat		
Fc .epsilon.RI	7	high (10.sup.-9-10.sup.-10 M)
Fc .gamma.RII	6 (7 total)	low
Fc .gamma.RIII	5	low
Other		
Fc .gamma.RII (guinea pig)	5 (6)	low
Fc .gamma.RIII (pig)	3	low
Fc .gamma.RII (bovine)	6	low

DETD [0141] In the high affinity ***FcRs*** , there are typically 5 to 7 potential N-linked glycosylation sites, whereas in the low affinity ***FcRs*** there are as few as two sites. One significant difference in the function of the high and low affinity ***FcRs*** is the probability that they will bind antibody in the absence of antigen. The high affinity receptors such as ***Fc*** .epsilon.RI can bind IgE prior to interacting with antigens. While not being bound by theory, it is believed that since ***FcR*** activation requires crosslinking of receptors, glycosylation might prevent the aggregation of large antibodies at the cell surface bound by ***FcRs*** . Crystallization of proteins at lipid/water interfaces can occur readily, and the potentially high local concentrations of membrane-bound antibodies might lead to ***FcR*** activation in the absence of antigen. The low affinity IgG receptors interact with antibody-antigen aggregates that can simultaneously bind and activate multiple ***FcRs*** . While not being bound by theory, it is believed that glycosylation may not be quite as important for these receptors,. . .

DETD [0142] However, it is likely that there are additional explanations for the glycosylation that is observed in the ***FcRs*** . The non-***human*** ***FcRs*** do not show an obvious correlation of the number of carbohydrate sites and ***FcR*** affinity. While not being bound by theory, it is believed that glycosylation might be important in ***FcR*** signaling, by orienting receptor:antibody complexes into functional signaling complexes (i.e. by preventing antigen-crosslinked complexes from forming non-functional aggregates). It is known that activation through ***Fc*** .epsilon.RI is sensitive to some geometrical constraints imposed by antigen crosslinkers, although the nature of these physical constraints is poorly understood.. . .

DETD [0144] A number of mutagenesis studies have been carried out in an effort to elucidate the regions of the ***FceRI*** that are critical for the interaction with IgE molecules (Cook et al., 1997, ibid.; Hulett et al., 1993, ibid.; Hulett. . . with the IgE or whether D1

indirectly alters the structure of D2 and subsequent interactions with IgE. Analysis of the ***Fc*** .epsilon.RI.alpha. model of the present invention is needed to predict important IgE binding regions in the protein. For example, the substitution or elimination of residues at the D1D2 interface can influence D2 interactions with antibody ***Fc*** regions.

DETD . . . of regions of D1 which have been excluded as determinants of the specificity of the receptor for IgE, since these ***Fc*** .epsilon.RI.alpha. segments can be substituted by the corresponding residues in the ***FcgRIIIA*** protein (Mallamaci et al., 1993, ibid.). These residues include segments 25-38, 43-54, 67-79, and 77-86. Substitution of residues 10-21 or. . .

DETD [0146] The ***Fc*** .epsilon.RI.alpha. residues which have been implicated in past studies include residues in the D2 BC loop (amino acid 115), in the. . . al., 1997, ibid.). Furthermore, a synthetic peptide corresponding to residues 119-129 has been demonstrated to block IgE binding to the ***Fc*** .epsilon.RI.alpha., with an apparent K.sub.D of about 3 mM (McDonnell et al., 1997, ibid.; McDonnell et al., 1996, ibid.).

DETD . . . residues. The remaining residues (i.e., amino acids 87, 117, 121, 123, 128, 159) are all exposed amino acids at the ***Fc*** .epsilon.RI.alpha. surface. All of the implicated residues form a contiguous surface extending from the back side of the D2 domain to the top region near the D1D2 interface. Four of the residues are conserved in all ***human*** ***FcRs*** (i.e., amino acids 87, 118, 149, and 153) and may define a set of common interactions between all ***FcR*** receptors and their target Ig molecules.

DETD . . . mutagenesis also borders on a number of surface accessible aromatic residues, including four prominent tryptophans at the top of the ***Fc*** .epsilon.RI.alpha. molecule, namely residues 87, 110, 113, and 156. These four tryptophans form a flat, hydrophobic ridge that neighbors the D2. . . and tryptophan 156 is prominently displayed at the top of the FG loop. Tryptophan 156 is a glycine in all ***FcgRs*** and grafting of residues 154-161 of the ***Fc*** .epsilon.RI.alpha. FG-loop to ***FcgRII*** confers IgE binding. It is to be noted, however, that such a graft does not eliminate IgG binding. The hFc.epsilon.RI.alpha.. . .

DETD [0149] E. Implications for the Binding of Other ***FcRs***

DETD . . . receptor surface as defined by models of the present invention. The positions of the carbohydrate attachment sites for seventeen related ***FcRs*** indicated that the N-linked carbohydrate sites delineate a boundary around the proposed IgE binding site. This is consistent with the suggestion that related ***FcRs*** share a common binding surface for antibody molecules. Studies of the ***FcgRII*** specificity for IgG, for example, have implicated the following residues: amino acids 113-116, 129, 131, 133, 134, 155, 156, and. . . et al., 1994, ibid.; Hulett et al., 1995, ibid.). In addition, domain-swap experiments have demonstrated that two of the related ***FcgRs*** can form functional hybrid molecules with ***Fc*** .epsilon.RI.alpha. (Hulett et al., 1995, ibid.; Mallamaci et al., 1993, ibid.), suggesting that these receptors share a common binding surface with. . . respective antibody ligands. Once again, however, it should be noted that only with the model can one predict exactly which ***FcR*** residues directly interact with an ***Fc*** domain of an antibody.

DETD [0151] The hFc.epsilon.RI.alpha. model indicates that the top of the ***FcR*** structure is devoid of carbohydrate-attachment sites in the region of D2 that has been implicated in direct interactions with Ig. . . binding site across the D1D2 interface. If these D1 loops are important in determining the specificity and affinity of the ***FcR*** :antibody interaction, one might observe sequence variability between high affinity and low IgG receptors and the IgE receptor. This variability in the ***human*** IgG and IgE receptors is shown in FIG. 5. For residues 3-173 of the hFc.epsilon.RI.alpha. protein, there are 73 amino. . . of the IgG receptors and these are indicated below the sequence alignments. Of these 73 amino acids unique to the ***human*** ***Fc*** .epsilon.RI.alpha. protein, 27 positions are highly variable in the different ***FcR*** sequences (>4/7 different amino acids. There are five regions that stand out with clusters of variable residues:residues 27-30, 47-49, 54-59,. . . 94-98 and 155-159. Residues 155-159 (the FG loop) are highly variable and do at

least partially determine the specificity of ***FcR*** interactions. It is again to be noted that without the model one cannot determine which regions of sequence variability are important in determining ***FcR*** protein functional domains.

DETD [0152] Previous experiments have shown that residues 27-30 and 47-49 are not critical for ***FcR*** specificity (Mallamaci et al., 1993, ibid.), and the presence of glycosylation sites within these segments in related ***FcRs*** support the suggestion that these regions are not part of the ***FcR*** :antibody interaction. The hFc.epsilon.RI.alpha. model indicates that residues 94-98 are found in the A' strand near the D1D2 cleft and therefore. . .

DETD [0153] The remaining group of highly variable residues (54-59) are in the D1 E strand (see FIG. 7), near the ***Fc*** .epsilon.RI.alpha. binding site as predicted by the hFc.epsilon.RI.alpha. model. Residues 54-59 could form a D1 surface of interaction with the ***Fc*** domains of antibodies, extending the binding site across both ***Fc*** .epsilon.RI.alpha. domains. This prediction is supported by a study reporting that the exchange of ***Fc*** .epsilon.RI.alpha. residues 55-67 with residues from the ***FcgRIIIA*** receptor disrupts the folding of the protein (Mallamaci et al., 1993, ibid.), as some of the residue changes form part. . .

DETD [0154] F. Stoichiometry of Binding Between ***FcR*** and Antibody

DETD [0155] The activation of ***FcR*** -bearing cells requires crosslinking of the receptors, which leads to the activation of intracellular kinase cascades analogous to those in B and T cells. For both high and low high affinity receptors ***FceRI*** and ***FcgRIII*** , a stoichiometry of 1:1 is observed between the receptor and the ***Fc*** domains of the respective antibodies to which they bind (Ghirlando et al., 1995, Biochemistry 34, 13320-13327; Kanellopoulos et al., 1980,. . . J. 25, 471-476), consistent with a requirement for antigen to cause receptor aggregation and activation. The binding site on the ***Fc*** domain of an IgE antibody for its receptor has been extensively studied by mutagenesis, implicating amino acids in the third. . . et al., 1991, Embo J. 10, 101-107; Presta et al., 1994, J. Biol. Chem. 269, 26368-26373). The structure of the ***Fc*** domain of IgE antibodies (also referred to as IgE- ***Fc*** domains) has not been experimentally determined, but is homologous to the ***Fc*** domain of IgG antibodies (also referred to as IgG- ***Fc*** domains), for which a number of crystal structures are available (Harris et al., 1998, J. Mol. Biol. 275, 861-872; Huber et al., 1976, Nature 264, 415-420). The residues of the IgE- ***Fc*** domain implicated in binding to ***FceRs*** based on mutagenesis analysis are shown mapped onto the structure of the IgG- ***Fc*** domain in FIG. 8. The site on an IgG- ***Fc*** domain to which ***FcgRI*** and ***FcgRII*** receptors bind has been mapped to a similar, although smaller, surface that primarily includes residues in the hinge region before. . .

DETD [0156] An antibody ***Fc*** domain is a homodimeric structure that is significantly larger than its respective ***FcR*** ; see FIG. 8. The observed 1:1 stoichiometry between receptor and antibody indicates that the two-fold symmetry of the ***Fc*** domain does not lead to the binding of two ***FcRs*** , even with isolated molecules in solution. Without being bound by theory, it is believed that the large and convex nature of the ***FcR*** binding surface could span two antibody domains (Cg2 in IgG and Ce3 in IgE) and induce a conformational change in the ***Fc*** domain that would prevent the binding of a second ***FcR*** to the same antibody. The ***FcR*** structure could also form an asymmetric complex with the antibody that sterically blocks the binding of a second ***FcR*** , perhaps using the protruding FG loop to block symmetric interactions with the ***Fc*** hinge region.

DETD [0157] This Example describes the production of additional three-dimensional models of the present invention as well as descriptions of ***Fc*** .epsilon.RI.alpha. proteins predicted therefrom.

DETD . . . 130.02

1507 C6 NAG A 274 53.668 -23.483 0.076 1.00 139.67

1508 O6 NAG A 274 52.628 -22.635 -0.387 1.00 144.24

1509 C1 ***FCA*** A 275 59.339 -23.324 1.916

```
      1.00   166.18
1510          C2          ***FCA***   A       275    59.905   -22.923  3.289
      1.00   176.23
1511          C3          ***FCA***   A       275    60.762   -21.695  3.251
      1.00   178.46
1512          C4          ***FCA***   A       275    61.991   -21.833  2.283
      1.00   172.76
1513          C5          ***FCA***   A       275    61.389   -22.191  0.896
      1.00   176.96
1514          C6          ***FCA***   A       275    62.389   -22.620  -0.196
      1.00   180.24
1515          O2          ***FCA***   A       275    58.772   -22.721  4.211
      1.00   187.28
1516          O3          ***FCA***   A       275    61.269   -21.376  4.553
      1.00   178.44
1517          O4          ***FCA***   A       275    62.910   -22.849  2.754
      1.00   169.66
1518          O5          ***FCA***   A       275    60.459   -23.282  0.968
      1.00   169.81
1519          C1        NAG A       276    57.235   -25.594  1.103     1.00
      98.00
1520          C2        NAG A       276.   .   .
DETD  .   .   .  97.83
3067          C6        NAG B       336    18.956   25.835   18.400    1.00
      97.91
3068          O6        NAG B       336    18.216   25.798   17.193    1.00
      97.89
3069          C1          ***FCA***   B       337    11.537   27.883   23.223
      1.00   97.62
3070          C2          ***FCA***   B       337    10.367   28.129   24.189
      1.00   97.53
3071          C3          ***FCA***   B       337    9.202    28.823   23.571
      1.00   97.82
3072          C4          ***FCA***   B       337    9.595    30.213   22.961
      1.00   97.76
3073          C5          ***FCA***   B       337    10.713   29.895   21.926
      1.00   97.77
3074          C6          ***FCA***   B       337    11.421   31.110   21.306
      1.00   97.74
3075          O2          ***FCA***   B       337    9.934    26.823   24.727
      1.00   97.69
3076          O3          ***FCA***   B       337    8.162    29.022   24.541
      1.00   97.99
3077          O4          ***FCA***   B       337    10.062   31.100   24.005
      1.00   97.77
3078          O5          ***FCA***   B       337    11.775   29.137   22.508
      1.00   97.61
3079          C1        NAG B       340    -0.412   38.735   24.336    1.00
      122.51
3080          C2        NAG B       340.   .   .
DETD  .   .   .  NAG        10.4998   0.0000          10.4998
AAAA       243        NAG          9.1915    0.0000          9.1915
AAAA       244        MAN         17.0951    0.0000         17.0951
AAAA       274        NAG          8.2536    0.0000          8.2536
AAAA       275          ***FCA***            13.5116   0.0000          13.5116
AAAA       276        NAG         18.0492    0.0000         18.0492
AAAA       340        NAG         18.2117    0.0000         18.2117
AAAA       366        NAG         20.2201    0.0000         20.2201
BBBB       1          VAL.   .   .
DETD       .   .   .  NAG        10.3502   0.0000          10.3502
BBBB       244        MAN         15.8885    0.0000         15.8885
BBBB       335        NAG          8.8279    0.0000          8.8279
BBBB       336        NAG         16.5384    0.0000         16.5384
BBBB       337          ***FCA***            16.2107   0.0000          16.2107
BBBB       340        NAG         13.5916    0.0000         13.5916
BBBB       341        NAG         21.2819    0.0000         21.2819
BBBB       366        NAG         21.9238    0.0000         21.9238
CLM    What is claimed is:
 .   .   .  1. A three-dimensional model selected from the group consisting of:
       (a) a three-dimensional model of an extracellular domain of a
         ***human***   high affinity   ***Fc***     ***epsilon***
```

receptor alpha chain (***Fc*** .epsilon.RI.alpha.) protein, wherein said model substantially represents the atomic coordinates specified in a table selected from the group consisting of Table. . . three-dimensional model comprising a modification of said model of (a), wherein said modification represents a protein that binds to a ***Fc*** domain of an antibody.

4. The model of claim 1, wherein said model represents a protein that binds to a ***Fc*** domain of an IgE antibody with an affinity that is at least equivalent to the affinity of the extracellular domain of ***human*** ***Fc*** .epsilon.RI.alpha. for an IgE antibody selected from the group consisting of a ***human*** IgE antibody, a canine IgE antibody, a feline IgE antibody, an equine IgE antibody, a rat IgE antibody, and a. . .

. . . of claim 1, wherein said model represents an extracellular domain of a protein selected from the group consisting of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, a canine ***Fc*** .epsilon.RI.alpha. protein, a feline ***Fc*** .epsilon.RI.alpha. protein, an equine ***Fc*** .epsilon.RI.alpha. protein, a murine ***Fc*** .epsilon.RI.alpha. protein, and a rat ***Fc*** .epsilon.RI.alpha. protein.

. . . wherein said model comprises a three-dimensional model of an extracellular antibody binding domain of an antibody receptor protein other than ***human*** ***Fc*** .epsilon.RI.alpha..

. . . the amino acid sequence of said other antibody receptor protein into a three-dimensional model of said extracellular domain of said ***human*** ***Fc*** .epsilon.RI.alpha. protein to produce said model of said other antibody receptor protein.

. . . model of claim 1, wherein said model is produced by a method comprising: (a) crystallizing an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein; (b) collecting X-ray diffraction data from said crystallized protein; and (c) determining said model from said data and amino. . .

. . . wherein said modification has an amino acid sequence that shares at least about 30% amino acid sequence homology with a ***Fc*** .epsilon.RI.alpha. protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO:. . .

14. The model of claim 1, wherein said model represents a ***Fc*** .epsilon.RI.alpha. protein having increased stability compared to the stability of a ***human*** ***Fc*** .epsilon.RI.alpha. protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO:. . .

15. The model of claim 1, wherein said model represents a ***Fc*** .epsilon.RI.alpha. protein having increased affinity for IgE compared to the affinity of a ***human*** ***Fc*** .epsilon.RI.alpha. protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO:. . .

16. The model of claim 1, wherein said model represents a ***Fc*** .epsilon.RI.alpha. protein having altered substrate affinity compared to the affinity of a ***human*** ***Fc*** .epsilon.RI.alpha. protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO:. . .

17. The model of claim 1, wherein said model comprises a three-dimensional model of a ***Fc*** .epsilon.RI.alpha. protein having increased solubility compared to the solubility of a ***human*** ***Fc*** .epsilon.RI.alpha. protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO:. . .

. . . The model of claim 1, wherein said model is used to identify an inhibitor of the selective binding between a ***Fc*** .epsilon.RI.alpha. protein and an IgE antibody.

19. The model of claim 1, wherein said model identifies crystal contacts between a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** domain of an IgE antibody.

22. A method to produce a three-dimensional model of an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, said

method comprising representing amino acids of said protein at substantially the atomic coordinates specified in a table selected. .

24. A method to produce a three-dimensional model of an antibody receptor protein other than a ***human*** ***Fc*** .epsilon.RI.alpha. protein represented by the three-dimensional model substantially representing the atomic coordinates specified in a table selected from the group consisting. . .

27. An isolated crystal of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein.

34. A method to produce an isolated crystal of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein, said method comprising vapor diffusion.

39. An isolated ***Fc*** .epsilon.RI.alpha. protein selected from the group consisting of: (a) a protein consisting of SEQ ID NO: 2; (b) a protein consisting. . . protein that is structurally homologous to a protein of (a) or (b), wherein said protein of (c) binds to a ***Fc*** domain of an antibody.

41. The protein of claim 39, wherein said ***Fc*** .epsilon.RI.alpha. protein is selected from the group consisting of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, a feline ***Fc*** .epsilon.RI.alpha. protein, a canine ***Fc*** .epsilon.RI.alpha. protein, an equine ***Fc*** .epsilon.RI.alpha. protein, a murine ***Fc*** .epsilon.RI.alpha. protein, and a rat ***Fc*** .epsilon.RI.alpha. protein.

47. A method to identify a compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein, said method comprising using a three-dimensional model of an extracellular domain of a ***human*** high affinity ***Fc*** .epsilon.RI.alpha. protein to identify said compound, wherein said model substantially represents the atomic coordinates specified in a table selected from the. . . . wherein such an interaction indicates that said compound is capable of inhibiting said binding of an IgE antibody to a ***Fc*** .epsilon.RI.alpha. protein.

. . . of identifying one or more amino acid(s) in the IgE binding domain of said model that interact directly with the ***Fc*** domain of an IgE antibody when said ***Fc*** domain binds to said IgE binding domain.

59. A mutein that binds to a ***Fc*** domain of an antibody, wherein said mutein has an improved function compared to a protein comprising an amino acid sequence. . . ID NO: 4, wherein said improved function is selected from the group consisting of increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, and increased solubility, wherein said mutein is produced by a method comprising: (a). . .

63. The mutein of claim 59, wherein said mutein has a K.sub.A for said ***Fc*** domain of at least about 3.times.10.sup.9 liters/mole.

64. The mutein of claim 59, wherein said mutein has a k.sub.a for said ***Fc*** domain of at least about 1.times.10.sup.5 liters/mole-second.

65. The mutein of claim 59, wherein said mutein has a k.sub.d for said ***Fc*** domain of less than or equal to 3.times.10.sup.-5/second.

. . . at least one amino acid segment of said antibody receptor protein with said improved function that if incorporated into said ***Fc*** .epsilon.RI.alpha. protein represented by said model would give said ***Fc*** .epsilon.RI.alpha. protein said improved function; and (b) incorporating said segment into said ***Fc*** .epsilon.RI.alpha. protein, thereby producing a mutein with said improved function.

. . . mutein is produced by a method comprising: (a) effecting random mutagenesis of nucleic acid molecules encoding a target of a ***Fc*** .epsilon.RI.alpha. protein as identified by analyzing a model of that protein; (b) cloning said mutagenized nucleic acid molecules into a phage. . .

. . . at least one amino acid in at least one non-constrained loop of domain 1 in an area proximal to the ***FceRI*** gamma chain putative binding site; (b) joining an amino-terminal amino acid residue to a carboxyl-terminal amino acid residue of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein; (c) replacing at least one amino acid site with an amino acid suitable for derivatization; (d) replacing at least. . .

81. A mutein having an improved function compared to an unmodified ***Fc*** .epsilon.RI.alpha. protein, wherein said improved function is selected from the group consisting of increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, and increased solubility, wherein the amino acid sequence of said mutein differs in. . .

82. A method to improve a function of a ***Fc*** .epsilon.RI.alpha. protein, said improved function being selected from the group consisting of increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, and increased solubility, said method comprising: (a) analyzing a three-dimensional model of an extracellular domain of a ***human*** high affinity ***Fc*** .epsilon.RI.alpha. protein substantially representing the atomic coordinates specified in a table selected from the group consisting of Table 1, Table 5,. . .

83. An isolated ***Fc*** .epsilon.RI.alpha. protein selected from the group consisting of: a crystal contact cluster involved in IgE binding; a tryptophan-containing hydrophobic ridge; a. . .

L13 ANSWER 5 OF 24 USPATFULL on STN
AN 2003:334703 USPATFULL
TI Anti-equine ***Fc*** ***epsilon*** ***receptor*** alpha chain antibodies and method to detect IgE
IN Weber, Eric R., Fort Collins, CO, UNITED STATES
McCall, Catherine A., Boulder, CO, UNITED STATES
PI US 2003235579 A1 20031225
AI US 2003-434817 A1 20030508 (10)
RLI Division of Ser. No. US 2000-515311, filed on 29 Feb 2000, GRANTED, Pat. No. US 6582701 Division of Ser. No. US 1998-15734, filed on 29 Jan 1998, GRANTED, Pat. No. US 6057127
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 101
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 3011
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect IgE using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.
TI Anti-equine ***Fc*** ***epsilon*** ***receptor*** alpha chain antibodies and method to detect IgE
AB The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . . such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.
SUMM [0001] The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . .
SUMM [0003] Immunological stimulation can be mediated by IgE antibodies when IgE complexes with ***Fc*** ***epsilon*** ***receptors*** .

Fc ***epsilon*** ***receptors*** are found on the surface of certain cell types, such as mast cells. Mast cells store biological mediators including histamine, prostaglandins and proteases. Release of these biological mediators is triggered when IgE antibodies complex with ***Fc*** ***epsilon*** ***receptors*** on the surface of a cell. Clinical symptoms result from the release of the biological mediators into the tissue of. . .

SUMM [0004] The discovery of the present invention includes a novel equine ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) alpha chain protein and the use of such a protein to detect the presence of IgE in a putative IgE-containing composition; to identify inhibitors of biological responses mediated by an equine ***Fc*** .sub..epsilon.R protein; and as a therapeutic compound to prevent or treat clinical symptoms that result from equine ***Fc*** .sub..epsilon.R-mediated biological responses.

SUMM [0005] Prior investigators have disclosed the nucleic acid sequence for: the ***human*** ***Fc*** .sub..epsilon.R alpha chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988; Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911, 1988; and Pang et al., J. Immunol. 151:6166-6174, 1993); the ***human*** ***Fc*** .sub..epsilon.R beta chain (Kuster et al., J. Biol. Chem. 267:12782-12787, 1992); the ***human*** ***Fc*** .sub..epsilon.R gamma chain (Kuster et al., J. Biol. Chem. 265:6448-6452, 1990); and the canine ***Fc*** .sub..epsilon.R alpha chain (GenBank.TM. accession number D16413). Although the subunits of ***human*** ***Fc*** .sub..epsilon.R have been known as early as 1988, they have never been used to identify an equine ***Fc*** .sub..epsilon.R. Similarly, even though the canine ***Fc*** .sub..epsilon.R chain has been known since 1993, it has never been used to identify an equine ***Fc*** .sub..epsilon.R. Moreover, the determination of ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** sequences does not indicate, suggest or predict the cloning of a novel ***Fc*** .sub..epsilon.R gene from a different species, in particular, from an equine species. Previous investigators have found a low degree of similarity between rat, mouse and ***human*** ***Fc*** .sub..epsilon.R.alpha. (Ravtech et al., Ann. Rev. Immunol. Vol. 9, pp. 457-492, 1991). Thus, given this low degree of sequence similarity, it would appear only "obvious to try" to obtain an equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule and protein.

SUMM . . . invention are needed in the art that will provide specific detection of IgE, in particular equine IgE, and treatment of ***Fc*** ***epsilon*** ***receptor*** -mediated disease.

SUMM [0007] The present invention relates to a novel product and process for detecting IgE and protecting animals from ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. According to the present invention there are provided equine ***Fc*** .sub..epsilon.R proteins and mimetopes thereof; equine ***Fc*** .sub..epsilon.R nucleic acid molecules, including those that encode such proteins; antibodies raised against such equine ***Fc*** .sub..epsilon.R proteins (i.e., anti-equine ***Fc*** .sub..epsilon.R antibodies); and other compounds that inhibit the ability of equine ***Fc*** .sub..epsilon.R protein to form a complex with IgE (i.e, inhibitory compounds or inhibitors).

SUMM . . . comprising such proteins, mimetopes, nucleic acid molecules, antibodies, and/or inhibitory compounds, as well as use of such therapeutic compositions to ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM [0009] One embodiment of the present invention is an isolated nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R protein. The equine ***Fc*** .sub..epsilon.R protein preferably includes: proteins comprising amino acid sequences SEQ ID NO: 2, SEQ ID NO: 7 and SEQ ID NO:. . . encoded by allelic variants of nucleic acid molecules encoding a protein comprising any of amino acid sequences. Particularly preferred equine ***Fc*** .sub..epsilon.R nucleic acid molecules include: nucleic acid molecules comprising nucleic acid sequences SEQ ID NO: 1, SEQ ID NO: 3, SEQ. . .

SUMM [0010] The present invention also includes an isolated equine ***Fc*** .sub..epsilon.R protein. A preferred equine ***Fc*** .sub..epsilon.R protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid sequence including SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 8. Particularly preferred equine ***Fc*** .sub..epsilon.R proteins include at least one of the

following amino acid sequences: SEQ ID NO: 2, SEQ ID NO: 7 and. . .

SUMM [0011] The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include equine ***Fc*** .sub..epsilon.R nucleic acid molecules of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant. . .

SUMM . . . detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon.R molecule:IgE complex, the presence of the ***Fc*** .sub..epsilon.R molecule:IgE complex indicating the presence of IgE. A preferred equine ***Fc*** .sub..epsilon.R molecule is one in which a carbohydrate group of the equine ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

SUMM . . . IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell comprises an equine ***Fc*** .sub..epsilon.R molecule; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred method to detect IgE comprises: (a) immobilizing the ***Fc*** .sub..epsilon.R molecule on a substrate; (b) contacting the ***Fc*** .sub..epsilon.R molecule with the putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .sub..epsilon.R molecule:IgE complex. Another preferred method to detect IgE comprises: (a) immobilizing a specific antigen on a substrate; (b) contacting the. . . binding to said substrate; and (d) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with said ***Fc*** .sub..epsilon.R molecule. Another preferred method to detect IgE comprises: (a) immobilizing an antibody that binds selectively to IgE on a substrate;. . . binding to the substrate; and (d) detecting the presence of the antibody:IgE complex by contacting the antibody:IgE complex with said ***Fc*** .sub..epsilon.R molecule. Another preferred method to detect IgE comprises: (a) immobilizing a putative IgE-containing composition on a substrate; (b) contacting the composition with the ***Fc*** .sub..epsilon.R molecule under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .sub..epsilon.R molecule:IgE complex.

SUMM . . . a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising an equine ***Fc*** .sub..epsilon.R protein and a means for detecting IgE.

SUMM [0015] The present invention also includes an inhibitor that interferes with formation of a complex between equine ***Fc*** .sub..epsilon.R protein and IgE, in which the inhibitor is identified by its ability to interfere with the complex formation. A particularly preferred inhibitor includes a substrate analog of an equine ***Fc*** .sub..epsilon.R protein, a mimetope of an equine ***Fc*** .sub..epsilon.R protein and a soluble portion of an equine ***Fc*** .sub..epsilon.R protein. Also included is a method to identify a compound that interferes with formation of a complex between equine ***Fc*** .sub..epsilon.R protein and IgE, the method comprising: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the equine ***Fc*** .sub..epsilon.R protein forms a complex with IgE; and (b) determining if the putative inhibitory compound inhibits the complex formation. A test kit is also included to identify a compound capable of interfering with formation of a complex between an equine ***Fc*** .sub..epsilon.R protein and IgE, the test kit comprising an isolated equine ***Fc*** .sub..epsilon.R protein that can complex with IgE and a means for determining the extent of interference of the complex formation in. . .

SUMM [0016] Yet another embodiment of the present invention is a therapeutic

composition that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. Such a therapeutic composition includes one or more of the following therapeutic compounds: an isolated equine ***Fc*** .sub..epsilon.R protein; a mimetope of an equine ***Fc*** .sub..epsilon.R protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R protein; and an inhibitor that interferes with formation of a complex between an equine ***Fc*** .sub..epsilon.R protein and IgE. A method of the present invention includes the step of administering to an animal a therapeutic composition. . .

SUMM [0017] Yet another embodiment of the present invention is a method to produce an equine ***Fc*** .sub..epsilon.R protein, the method comprising culturing a cell transformed with a nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R protein.

SUMM [0018] The present invention provides for isolated equine ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .sub..epsilon.R.alpha.) proteins, isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules, antibodies directed against equine ***Fc*** .sub..epsilon.R.alpha. proteins and other inhibitors of equine ***Fc*** .sub..epsilon.R.alpha. activity. As used herein, the terms isolated equine ***Fc*** .sub..epsilon.R.alpha. proteins and isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules refers to ***Fc*** .sub..epsilon.R.alpha. proteins and ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules derived from horses and, as such, can be obtained from their natural source or can be produced. . . of the present invention are advantageous because they enable the detection of IgE and the inhibition of IgE or equine ***Fc*** .sub..epsilon.R.alpha. protein activity associated with disease. As used herein, equine ***Fc*** epsilon alpha chain receptor protein can be referred to as ***Fc*** .sub..epsilon.R.alpha. protein or ***Fc*** .sub..epsilon.R alpha chain protein.

SUMM [0019] One embodiment of the present invention is an isolated protein comprising an equine ***Fc*** .sub..epsilon.R.alpha. protein. It is to be noted that the term "a" or "an" entity refers to one or more of that. . .

SUMM [0020] As used herein, an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein can be a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. Preferably, an equine ***Fc*** .sub..epsilon.R.alpha. protein comprises at least a portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE, i.e., that is capable of forming a complex with an IgE.

SUMM [0021] An equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to IgE. Examples of equine ***Fc*** .sub..epsilon.R.alpha. protein homologs include equine ***Fc*** .sub..epsilon.R.alpha. proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted,. . .

SUMM [0022] Equine ***Fc*** .sub..epsilon.R.alpha. protein homologs can be the result of natural allelic variation or natural mutation. Equine ***Fc*** .sub..epsilon.R.alpha. protein homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

SUMM [0023] Isolated equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention have the further characteristic of being encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to a gene encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are. . .

SUMM [0024] As used herein, an equine ***Fc*** .sub..epsilon.R.alpha. gene includes all nucleic acid sequences related to a natural equine ***Fc*** .sub..epsilon.R.alpha. gene such as regulatory regions that control production of the equine ***Fc*** .sub..epsilon.R.alpha. protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In one embodiment, an equine ***Fc***

.sub..epsilon.R.alpha. gene of the present invention includes nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO:. . .

SUMM . . . other nucleic acid and protein sequences presented herein) represent apparent nucleic acid sequences of certain nucleic acid molecules encoding equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention.

SUMM [0026] In another embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. gene can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO: 1, SEQ. . . 5, SEQ ID NO: 6, SEQ ID NO: 8 and/or SEQ ID NO: 11. An allelic variant of an equine ***Fc*** .sub..epsilon.R.alpha. gene is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including. . .

SUMM [0027] The minimal size of a ***Fc*** .sub..epsilon.R.alpha. protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of. . . length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode an equine ***Fc*** .sub..epsilon.R.alpha. protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of an equine ***Fc*** .sub..epsilon.R.alpha. protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no. . .

SUMM . . . used herein, an equine refers to any member of the horse family. Examples of horses from which to isolate equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention (including isolation of the natural protein or production of the protein by recombinant or synthetic. . .

SUMM [0029] Suitable horse cells from which to isolate an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention include cells that have ***Fc*** .sub..epsilon.R.alpha. proteins. Preferred horse cells from which to obtain an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention include basophil cells, mast cells, mastocytoma cells, dendritic cells, B lymphocytes, macrophages, eosinophils, and/or monocytes. An equine ***Fc*** .sub..epsilon.R.alpha. of the present invention is preferably obtained from mastocytoma cells, mast cells or basophil cells.

SUMM [0030] The present invention also includes mimetopes of equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention. As used herein, a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention refers to any compound that is able to mimic the activity of such an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., ability to bind to IgE), often because the mimetope has a structure that mimics the equine ***Fc*** .sub..epsilon.R.alpha. protein. It is to be noted, however, that the mimetope need not have a structure similar to an equine ***Fc*** .sub..epsilon.R.alpha. protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to: peptides that. . . nucleic acids; and/or any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides. . . other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., an equine IgE ***Fc*** domain or anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the. . . for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of equine ***Fc*** .sub..epsilon.R.alpha. mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex.TM. technology, peptides identified by random screening of peptide libraries and proteins identified. . . by phage display technology. A preferred mimetope is a peptidomimetic compound that is structurally and/or functionally similar to an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, particularly to the IgE ***Fc*** domain binding site of the equine ***Fc*** .sub..epsilon.R.alpha. protein. As used herein, the ***Fc*** domain of an antibody refers to the portion of an immunoglobulin that has ***Fc*** receptor binding effector function. Typically, the ***Fc*** domain of an IgE comprises the CH2 and CH3 domains of the heavy chain constant region.

SUMM [0031] According to the present invention, an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention refers to: an equine ***Fc*** .sub..epsilon.R.alpha. protein, in particular a soluble equine ***Fc*** .sub..epsilon.R.alpha. protein; an equine ***Fc*** .sub..epsilon.R.alpha. homolog; an equine ***Fc*** .sub..epsilon.R.alpha. mimetope; an equine ***Fc*** .sub..epsilon.R.alpha. substrate analog; or an equine ***Fc*** .sub..epsilon.R.alpha. peptide. Preferably, an equine ***Fc*** .sub..epsilon.R.alpha. molecule binds to IgE.

SUMM [0032] One embodiment of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention is a fusion protein that includes an equine ***Fc*** .sub..epsilon.R.alpha. protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but. . . to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against an equine ***Fc*** .sub..epsilon.R.alpha. protein; and/or assist purification of an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired. . . protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the equine ***Fc*** .sub..epsilon.R.alpha.-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of an equine ***Fc*** .sub..epsilon.R.alpha. protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of an equine ***Fc*** .sub..epsilon.R.alpha.-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . . the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline phosphatase domain connected to an equine ***Fc*** .sub..epsilon.R.alpha. protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding. . .

SUMM [0033] A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the following nucleic acid molecules: neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 and neqFc.sub..epsilon.R.alpha..sub.603. Preferably, the equine ***Fc*** .sub..epsilon.R.alpha. protein binds to IgE. A further preferred isolated protein is encoded by a nucleic acid molecule that hybridizes under stringent. . .

SUMM . . . sequences reported in GenBank.TM. indicates that SEQ ID NO: 2 showed the most homology, i.e., about 61% identity, with a ***human*** high affinity IgE receptor .alpha.-subunit (SwissProt accession number P12319).

SUMM [0036] More preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention include proteins comprising amino acid sequences that are at least about 65%, preferably at least. . .

SUMM [0037] More preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention include proteins encoded by a nucleic acid molecule comprising at least a portion of neqFc.sub..epsilon.R.alpha..sub.1005,. . . allelic variants of such nucleic acid molecules, the portion being capable of binding to IgE. More preferred is an equine ***Fc*** .sub..epsilon.R.alpha. protein encoded by neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 and/or neqFc.sub..epsilon.R.alpha..sub.603, or by an allelic variant of such nucleic acid molecules. Particularly preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins are PequFc.sub..epsilon.R.alpha..sub.255, PequFc.sub..epsilon.R.alpha..sub.236 and PequFc.sub..epsilon.R.alpha..sub.201.

SUMM [0038] In one embodiment, a preferred equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention is encoded by at least a portion of SEQ ID NO: 1, SEQ ID NO:. . .

SUMM [0039] Also preferred is an equine ***Fc*** .sub..epsilon.R.alpha. protein encoded by an allelic variant of a nucleic acid molecule comprising at least a portion of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 6 and/or SEQ ID NO: 11. Particularly preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention include SEQ ID NO: 2, SEQ ID NO: 7 and SEQ ID NO: 12 (including,. . .

SUMM . . . embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R.alpha. gene. The identifying characteristics of such a gene are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural equine ***Fc*** .sub..epsilon.R.alpha. gene or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of. . . nucleic acid molecule of the present invention is the minimal size that can form a stable hybrid with an equine ***Fc*** .sub..epsilon.R.alpha. gene under stringent hybridization conditions.

SUMM . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule of the present invention can be isolated from its natural source or can be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions,. . . a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention or to form stable hybrids under stringent conditions with natural gene isolates.

SUMM [0042] An equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art (see,. . . mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with an equine ***Fc*** .sub..epsilon.R.alpha. gene or by screening for function of a protein encoded by the nucleic acid molecule (e.g., ability of an equine ***Fc*** .sub..epsilon.R.alpha. protein to bind equine IgE).

SUMM . . . isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers. . . interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein.

SUMM [0044] One embodiment of the present invention is an equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule that hybridizes under stringent hybridization conditions with nucleic acid molecule neqFc.sub..epsilon.R.alpha..sub.1015 and preferably with a nucleic acid. . .

SUMM . . . sequences reported in GenBank indicates that SEQ ID NO: 1 showed the most homology, i.e., about 75% identity to a ***human*** mRNA for immunoglobulin E receptor alpha chain gene (Accession number X06948).

SUMM [0046] Preferred equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules include nucleic acid molecules having a nucleic acid sequence that is at least about 80%, preferably at. . .

SUMM . . . ID NO: 6, SEQ ID NO: 8 and/or SEQ ID NO: 11, that is capable of hybridizing to an equine ***Fc*** .sub..epsilon.R.alpha. gene of the present invention, as well as allelic variants thereof. A more preferred nucleic acid molecule includes the nucleic. . .

SUMM [0048] Preferred equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules also include nucleic acid molecules having a nucleic acid sequence that is at least about 80%, preferably. . .

SUMM [0050] Knowing the nucleic acid sequences of certain equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of. . . (e.g., nucleic acid molecules including full-length genes, full-length

coding regions, regulatory control sequences, truncated coding regions), and (c) obtain equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules from other horses. Such nucleic acid molecules can be obtained in a variety of ways including screening. . .

SUMM . . . conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising equine ***Fc*** .sub..epsilon.R.alpha. genes or other equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of. . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit equine ***Fc*** .sub..epsilon.R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the. . .

SUMM . . . is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulation of equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules of the present invention.

SUMM . . . the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences. . .

SUMM . . . that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 . . . .

SUMM . . . production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention or can be capable of producing such proteins after being transformed with at least one. . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM [0063] Isolated equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins,. . . permit protein production. An effective medium refers to any medium in which a cell is cultured to produce an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources,. . .

SUMM [0065] The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention or a mimetope thereof (i.e., anti-equine ***Fc*** .sub..epsilon.R.alpha. antibodies). As used herein, the term "selectively binds to" an equine ***Fc*** .sub..epsilon.R.alpha. protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof. . . in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., ibid. An anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody preferably selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. protein in such a way as to reduce the activity of that protein.

SUMM . . . antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not. . .

SUMM . . . are within the scope of the present invention. For example, such antibodies can be used (a) as tools to detect ***Fc*** ***epsilon*** ***receptor*** in the presence or absence of IgE and/or (b) as tools to screen expression libraries and/or to recover desired proteins. . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic

agents to cells having ***Fc*** ***epsilon*** ***receptors*** such as those disclosed herein in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably. . . in the art. Suitable cytotoxic agents are known to those skilled in the art. Antibodies of the present invention, including ***Fc*** .sub..epsilon.R.alpha.-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** ***epsilon*** ***receptors*** , to produce anti-equine ***Fc*** .sub..epsilon.R.alpha. idiotypic antibodies, to purify cells having equine ***Fc*** .sub..epsilon.R.alpha. proteins, to stimulate intracellular signal transduction through an equine ***Fc*** .sub..epsilon.R.alpha. and to identify cells having equine ***Fc*** .sub..epsilon.R.alpha. proteins.

SUMM [0069] An equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of an equine ***Fc*** .sub..epsilon.R.alpha. molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon.R.alpha. molecule portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon.R.alpha. molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an. . .

SUMM [0070] An equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of an equine ***Fc*** .sub..epsilon.R.alpha. molecule that binds to an IgE and a second molecule, such as an enzyme, that enables the chimeric molecule to bind to IgE in essentially the same manner as a ***Fc*** .sub..epsilon.R.alpha. molecule which does not include such a second molecule, and to hydrolyze a substrate in such a manner so as. . . An example of a suitable second molecule includes alkaline phosphatase, horse radish peroxidase or urease. In one embodiment an equine ***Fc*** .sub..epsilon.R.alpha. chimeric molecule of the present invention comprises a protein encoded by a recombinant molecule comprising a nucleic acid molecule that encodes at least a portion of an equine ***Fc*** .sub..epsilon.R.alpha. molecule that binds to an IgE, operatively linked to a nucleic acid molecule that encodes an enzyme, preferably alkaline phosphatase.

SUMM [0071] An equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon.R.alpha. molecule formulation. For example, an equine ***Fc*** .sub..epsilon.R.alpha. molecule can be combined with a buffer in which the equine ***Fc*** .sub..epsilon.R.alpha. molecule is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which an equine ***Fc*** .sub..epsilon.R.alpha. molecule can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with equine ***Fc*** .sub..epsilon.R.alpha. molecules or conjugated (i.e., attached) to equine ***Fc*** .sub..epsilon.R.alpha. molecules in such a manner as to not substantially interfere with the ability of the equine ***Fc*** .sub..epsilon.R.alpha. molecules to selectively bind to IgE.

SUMM [0072] An equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention can be bound to the surface of a cell comprising the equine ***Fc*** .sub..epsilon.R.alpha. protein. A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein-bearing cell includes a recombinant cell comprising a nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention. A more preferred recombinant cell of the present invention comprises a nucleic acid molecule that. . .

SUMM [0073] In addition, an equine ***Fc*** .sub..epsilon.R.alpha. molecule formulation of the present invention can include not only an equine ***Fc*** .sub..epsilon.R.alpha. molecule but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

SUMM . . . of the present invention is a method to detect IgE which includes the steps of: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of an equine

Fc .sub..epsilon.R.alpha. molecule:IgE complex; and (b) detecting the presence of IgE by detecting the equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex. Presence of such an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex indicates that the animal is producing IgE. Preferred IgE to detect using an equine ***Fc*** .sub..epsilon.R.alpha. molecule include equine IgE, canine IgE, feline IgE and ***human*** IgE, with equine IgE being particularly preferred. The present method can further include the step of determining whether an IgE complexed with an equine ***Fc*** .sub..epsilon.R.alpha. protein is heat labile. Preferably, a heat labile IgE is determined by incubating an IgE at about 56.degree. C. for. . .

SUMM . . . used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with an equine ***Fc*** .sub..epsilon.R.alpha. molecule. Formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. molecule and an IgE refers to the ability of the equine ***Fc*** .sub..epsilon.R.alpha. molecule to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between an equine ***Fc*** .sub..epsilon.R.alpha. molecule and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between equine ***Fc*** .sub..epsilon.R.alpha. molecule and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the equine ***Fc*** .sub..epsilon.R.alpha. molecule or to a reagent that selectively binds to the equine ***Fc*** .sub..epsilon.R.alpha. molecule or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable*** ***marker*** can be connected to an equine ***Fc*** .sub..epsilon.R.alpha. molecule using, for example, chemical conjugation or recombinant DNA technology (e.g., connection of a fusion segment such as that described. . . binding domain; an immunoglobulin binding; a sugar binding domain; and a "tag" domain). Preferably a carbohydrate group of the equine ***Fc*** .sub..epsilon.R.alpha. molecule is chemically conjugated to biotin.

SUMM [0082] In one embodiment, a complex is detected by contacting a putative IgE-containing composition with an equine ***Fc*** .sub..epsilon.R.alpha. molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to an equine ***Fc*** .sub..epsilon.R.alpha. molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to an equine ***Fc*** .sub..epsilon.R.alpha. molecule in such a manner as not to block the ability of the equine ***Fc*** .sub..epsilon.R.alpha. molecule to bind to the IgE being detected. Preferably, a carbohydrate group of an equine ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to biotin.

SUMM [0083] In another embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting a putative IgE-containing composition with an equine ***Fc*** .sub..epsilon.R.alpha. molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the equine ***Fc*** .sub..epsilon.R.alpha. molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, an antigen, an antibody and a lectin, depending upon which portion of the equine

Fc .sub..epsilon.R.alpha. molecule:IgE complex is being detected. Preferred indicator molecules that are antibodies include, for example, anti-IgE antibodies and anti-equine ***Fc*** .sub..epsilon.R.alpha. antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

SUMM [0084] In one preferred embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with an indicator molecule that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention. Examples of such indicator molecule includes, but are not limited to, an antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. molecule (referred to herein as an anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to an equine ***Fc*** .sub..epsilon.R.alpha. molecule. An equine ***Fc*** .sub..epsilon.R.alpha. molecule conjugated to biotin is preferably detected using streptavidin.

SUMM [0085] In another preferred embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with indicator molecule that selectively binds to an IgE antibody (referred to. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. A preferred indicator molecule includes an anti-equine IgE antibody. As used herein, an anti-IgE. . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

SUMM [0088] A preferred immunoabsorbent assay method includes a step of either: (a) immobilizing an equine ***Fc*** .sub..epsilon.R.alpha. molecule on a substrate prior to contacting an equine ***Fc*** .sub..epsilon.R.alpha. molecule with a putative IgE-containing composition to form an equine ***Fc*** .sub..epsilon.R.alpha. molecule-immobilized substrate; and (b) binding a putative IgE-containing composition on a substrate prior to contacting an equine ***Fc*** .sub..epsilon.R.alpha. molecule with a putative IgE-containing composition to form a putative IgE-containing composition-bound substrate. Preferably, the substrate includes a non-coated substrate, an equine ***Fc*** .sub..epsilon.R.alpha. molecule-immobilized substrate, an antigen-immobilized substrate or an anti-IgE antibody-immobilized substrate.

SUMM . . . whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention is used as a capture molecule when the equine ***Fc*** .sub..epsilon.R.alpha. molecule is bound on a substrate. Alternatively, an equine ***Fc*** .sub..epsilon.R.alpha. molecule is used as an indicator molecule when the equine ***Fc*** .sub..epsilon.R.alpha. molecule is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

SUMM . . . the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an equine ***Fc*** .sub..epsilon.R.alpha. molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

SUMM [0092] In one embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule is used as a capture molecule by being immobilized on a

substrate, such as a microtiter dish well or. . . biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the equine ***Fc*** .sub..epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a calorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. '. .

SUMM . . . if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. An equine ***Fc*** .sub..epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the equine ***Fc*** .sub..epsilon.R.alpha. molecule and the anti-IgE antibody:IgE complex. Preferably, the equine ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess equine ***Fc*** .sub..epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. An equine ***Fc*** .sub..epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the equine ***Fc*** .sub..epsilon.R.alpha. molecule and the IgE. Preferably, the equine ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess equine ***Fc*** .sub..epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a calorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case an equine ***Fc*** .sub..epsilon.R.alpha. molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is. . .

SUMM . . . used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising an equine ***Fc*** .sub..epsilon.R.alpha. molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture. . .

SUMM . . . in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention and an isolated IgE known to bind to the equine ***Fc*** .sub..epsilon.R.alpha. molecule. The absence of binding of the equine ***Fc*** .sub..epsilon.R.alpha. molecule to the known IgE indicates the presence of IgE in the putative IgE-containing composition. The known IgE is preferably conjugated to a ***detectable*** ***marker*** .

SUMM . . . IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising an equine ***Fc*** .sub..epsilon.R.alpha. protein and a means for detecting an IgE. Suitable and preferred equine ***Fc*** .sub..epsilon.R.alpha. protein are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the equine ***Fc*** .sub..epsilon.R.alpha. protein or to an IgE. A preferred kit of the present invention further comprises a detection means including one or. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., avidin, streptavidin and

ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including equines, canines and/or felines.

SUMM . . . invention is a general allergen kit comprising an allergen common to all regions of the United States and an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are. . .

SUMM . . . allergen including wheat, corn, alfalfa, hay, straw, oats, grains, processed grain by-products and grasses and/or dusts thereof, and an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention. Kits for detecting hypersensitivity to feeds and/or feed dust allergens can be used in combination. . .

SUMM . . . present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of reducing ***Fc*** receptor mediated reactions associated with diseases related to biological responses involving ***Fc*** receptor function. A therapeutic composition of the present invention can include: an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein, or homolog thereof; a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R.alpha. gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. protein; and/or an inhibitor that interferes with formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE.

SUMM [0105] One embodiment of a therapeutic composition of the present invention is a therapeutic compound comprising an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention, that binds to an IgE. According to the present invention, an equine ***Fc*** .sub..epsilon.R.alpha. molecule competes for IgE with naturally-occurring ***Fc*** ***epsilon*** ***receptors*** , particularly those on mastocytoma cells, mast cells or basophils, so that IgE is bound to the administered equine ***Fc*** .sub..epsilon.R.alpha. molecule and thus is unable to bind to ***Fc*** ***epsilon*** ***receptor*** on a cell, thereby inhibiting mediation of a biological response. Preferred equine ***Fc*** .sub..epsilon.R.alpha. molecule for use in a therapeutic composition comprises an equine ***Fc*** .sub..epsilon.R.alpha. protein, or homolog thereof, as described herein, particularly a fragment thereof, which binds to IgE. Equine ***Fc*** .sub..epsilon.R.alpha. molecules for use in a therapeutic composition can be in a monovalent and/or multivalent form, so long as the equine ***Fc*** .sub..epsilon.R.alpha. molecule is capable of binding to IgE. A more preferred equine ***Fc*** .sub..epsilon.R.alpha. molecule for use in a therapeutic composition includes a soluble fragment of an equine ***Fc*** .sub..epsilon.R.alpha. protein. A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein is encoded by neqFc.sub..epsilon.R.alpha..sub.603 and an even more preferred equine ***Fc*** .sub..epsilon.R.alpha. protein is PequFc.sub..epsilon.R.alpha..sub.201.

SUMM . . . therapeutic composition of the present invention comprises a therapeutic compound that interferes with the formation of a complex between equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE, usually by binding to or otherwise interacting with or otherwise modifying the equine ***Fc*** .sub..epsilon.R.alpha. protein's IgE binding site. Equine ***Fc*** .sub..epsilon.R.alpha. protein inhibitors can also interact with other regions of the equine ***Fc*** .sub..epsilon.R.alpha. protein to inhibit equine ***Fc*** .sub..epsilon.R.alpha. protein activity, for example, by allosteric interaction. An inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. protein can interfere with ***Fc*** .sub..epsilon.R.alpha. protein and IgE complex formation by, for example, preventing formation of a ***Fc*** .sub..epsilon.R.alpha. protein and IgE complex or disrupting an existing ***Fc*** .sub..epsilon.R.alpha. protein and IgE complex causing the ***Fc*** .sub..epsilon.R.alpha. protein and IgE to dissociate. An inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. protein is usually a relatively small molecule. Preferably, an equine ***Fc*** .sub..epsilon.R.alpha. protein inhibitor of the present invention is identified by its ability to bind to, or otherwise interact with, an

equine ***Fc*** .sub..epsilon.R.alpha. protein, thereby interfering with the formation of a complex-between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE.

SUMM [0108] Preferred inhibitors of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention include, but are not limited to, a substrate analog of an equine ***Fc*** .sub..epsilon.R.alpha. protein, a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein, a soluble (i.e., secreted form of an equine ***Fc*** .sub..epsilon.R.alpha. protein) portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE, and other molecules that bind to an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., to an allosteric site) in such a manner that IgE-binding activity of the equine ***Fc*** .sub..epsilon.R.alpha. protein is inhibited. An equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the IgE-binding site of an equine ***Fc*** .sub..epsilon.R.alpha. protein. A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analog inhibits IgE-binding activity of an equine ***Fc*** .sub..epsilon.R.alpha. protein. Equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analogs can be of any inorganic or organic composition, and, as such, can be, but are not limited to, peptides, nucleic acids, and peptidomimetic compounds. Equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analogs can be, but need not be, structurally similar to an equine ***Fc*** .sub..epsilon.R.alpha. protein's natural substrate (e.g., IgE) as long as they can interact with the active site (e.g., IgE-binding site of that equine ***Fc*** .sub..epsilon.R.alpha.). Equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analogs can be designed using computer-generated structures of equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention or computer structures of, for example, the ***Fc*** domain of IgE. Substrate analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic. . . inorganic or organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., an equine ***Fc*** .sub..epsilon.R.alpha. protein or anti-equine ***Fc*** .sub..epsilon.R.alpha. idiotypic antibody). A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural substrate of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, particularly to the region of the substrate that binds to an equine ***Fc*** .sub..epsilon.R.alpha. protein, but that inhibits IgE binding upon interacting with the IgE binding site).

SUMM [0109] Equine ***Fc*** .sub..epsilon.R.alpha. molecules, as well as other inhibitors and therapeutic compounds, can be used directly as compounds in compositions of the present. . .

SUMM [0111] In one embodiment, a therapeutic composition of the present invention can be used to reduce a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response in an animal by administering such a composition to an animal. Preferably, an animal is treated by administering. . . therapeutic composition of the present invention in such a manner that a therapeutic compound (e.g., an inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. protein, an anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody, an inhibitor of IgE, or nucleic acid molecules encoding equine ***Fc*** .sub..epsilon.R.alpha. proteins) binds to an IgE or a ***Fc*** ***epsilon*** ***receptor*** in the animal. Such administration could be by a variety of routes known to those skilled in the art including,. . .

SUMM [0112] Compositions of the present invention can be administered to any animal having a ***Fc*** ***epsilon*** ***receptor*** or an IgE that binds to a therapeutic compound of the present invention or to a protein expressed by a. . .

SUMM . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the

animal. As used herein, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response refers to cellular responses that occur when ***Fc*** ***epsilon*** ***receptor*** is complexed with IgE. For example, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response includes release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . of skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen-specific ***Fc*** ***epsilon*** ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into an equine ***Fc*** .sub..epsilon.R.alpha. protein or an equine ***Fc*** .sub..epsilon.R.alpha. RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered. . .

SUMM . . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising an equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing an amount of protein or RNA sufficient to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. A preferred single dose of a recombinant virus of the present invention is from about 1.times.10.sup.4 to about. . .

SUMM . . . a therapeutic composition of the present invention includes recombinant cells of the present invention that comprises at least one equine ***Fc*** .sub..epsilon.R.alpha. of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda, yeast, (including. . .

SUMM . . . an animal an effective amount of a therapeutic composition selected from the group consisting of an inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. and an equine ***Fc*** .sub..epsilon.R.alpha. protein (including homologs), wherein said equine ***Fc*** .sub..epsilon.R.alpha. is capable of binding to IgE. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According to the. . . invention, a therapeutic composition and method of the present invention can be used to prevent or alleviate symptoms associated with ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM [0126] The efficacy of a therapeutic composition of the present invention to effect ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses can be tested using standard methods for detecting ***Fc*** receptor-mediated immunity including, but not limited to, immediate hypersensitivity, delayed hypersensitivity, antibody-dependent cellular cytotoxicity (ADCC), immune complex activity, mitogenic activity,. . .

SUMM [0127] An inhibitor of equine ***Fc*** .sub..epsilon.R.alpha. activity can be identified using equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention by determining the ability of an inhibitor to prevent or disrupt complex formation between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE. One embodiment of the present invention is a method to identify a compound capable of inhibiting equine ***Fc*** .sub..epsilon.R.alpha. activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the equine ***Fc*** .sub..epsilon.R.alpha. protein has IgE binding activity, and (b) determining if the putative inhibitory compound inhibits the IgE binding activity. Putative inhibitory. . .

SUMM [0128] The present invention also includes a test kit to identify a compound capable of inhibiting equine ***Fc*** .sub..epsilon.R.alpha. activity. Such a test kit includes: an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein having IgE binding activity or a complex of equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE; and a means for determining the extent of inhibition of IgE binding activity in the presence of. . .

DETD [0131] This example describes the isolation, by DNA hybridization, of a nucleic acid molecule encoding a ***Fc*** .sub..epsilon.R.alpha. chain from Equus caballus.

DETD . . . was isolated from a horse buffy coat cDNA library by its ability to hybridize with a .sup.32P-labeled cDNA encoding the ***human*** ***Fc*** .sub..epsilon.R.alpha. chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988). The horse buffy coat cDNA library was prepared as follows. Total. . .

DETD [0133] The horse buffy coat cDNA library was screened, using duplicate plaque filter lifts, with a .sup.32P-labeled cDNA encoding the ***human*** ***Fc*** .sub..epsilon.R.alpha. chain under the following conditions. The filters were pre-hybridized and hybridized in a hybridization solution including 5.times.SSC, 5.times. Denhardts, 0.5%. . .

DETD [0134] This example describes the sequencing of an equine ***Fc*** .sub..epsilon.R.alpha. chain nucleic acid molecule of the present invention.

DETD . . . includes GenBank+EMBL+DDBJ+PDB. The highest scoring match of the homology search at the amino acid level was SwissProt accession number P12319: ***human*** high affinity IgE receptor .alpha.-chain, which was about 61% identical with SEQ ID NO: 2. At the nucleotide level, the search was performed using SEQ ID NO: 1, which was most similar to GenBank accession number X06948, ***human*** mRNA for immunoglobulin E receptor alpha chain, which was about 75% identical to SEQ ID NO: 1.

DETD [0139] This Example demonstrates the production of an equine ***Fc*** .sub..epsilon.R.alpha. chain protein in eukaryotic cells.

DETD . . . SEQ ID NO: 1, operatively linked to baculovirus polyhedron transcription control sequences, was produced in the following manner. An equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule-containing fragment of about 603 nucleotides was PCR amplified from neqFc.sub..epsilon.R.alpha..sub.1015 using sense primer EqIgErFor having the nucleic. . .

DETD . . . referred to herein as pFB-neqFc.sub..epsilon.R.alpha..sub.603. Translation of SEQ ID NO: 11 indicates that the nucleic acid molecule neqFc.sub..epsilon.R.alpha..sub.603 encodes a ***Fc*** .sub..epsilon.R.alpha. protein of about 201 amino acids, referred to herein as PequFc.sub..epsilon.R.alpha..sub.201, having amino acid sequence SEQ ID NO: 12.

DETD . . . frugiperda; pFB-neqFc.sub..epsilon.R.alpha..sub.603 can be cultured using conditions known to those skilled in the art in order to produce the equine ***Fc*** .sub..epsilon.R.alpha. protein, PequFc.sub..epsilon.R.alpha..sub.201 or a secreted form thereof.

CLM What is claimed is:

1. An isolated nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein.

2. The nucleic acid molecule of claim 1, wherein said equine ***Fc*** .sub..epsilon.R.alpha. protein is selected from the group consisting of: a protein that comprises an amino acid sequence selected from the group. . .

13. An isolated equine ***Fc*** .sub..epsilon.R.alpha. protein.

19. The protein of claim 13, wherein said equine ***Fc*** .sub..epsilon.R.alpha. protein comprises a protein selected from the group consisting of PequFc.sub..epsilon.R.alpha..sub.255, PequFc.sub..epsilon.R.alpha..sub.236 and PequFc.sub..epsilon.R.alpha..sub.720.

21. A method to produce an equine ***Fc*** .sub..epsilon.R.alpha. protein, said method comprising culturing a cell transformed with a nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein.

25. A method to detect IgE comprising: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex; and (b) determining the presence of IgE by detecting said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex, the presence of said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex indicating the

presence of IgE.

26. The method of claim 25, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule comprises a protein selected from the group consisting of PequFc.sub..epsilon.R.alpha..sub.255, PequFc.sub..epsilon.R.alpha..sub.236 and PequFc.sub..epsilon.R.alpha..sub.201.

27. The method of claim 25, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule is encoded by a nucleic acid molecule selected from the group consisting of neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 and neqFc.sub..epsilon.R.alpha..sub.603.

28. The method of claim 25, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** .

29. The method of claim 25, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, an enzyme, a fluorescent label, a chemiluminescent label, a chromophoric label. . .

30. The method of claim 25, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, biotin-related compounds, avidin, avidin-related compounds and a peroxidase.

. . .

31. The method of claim 28, wherein said ***detectable*** ***marker*** is connected to said ***Fc*** .sub..epsilon.R.alpha. molecule by chemical conjugation or recombinant DNA technology.

32. The method of claim 25, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to biotin.

37. The method of claim 25, further comprising the step selected from the group consisting of immobilizing said ***Fc*** .sub..epsilon.R.alpha. molecule on a substrate prior to performing step (a) to form a ***Fc*** .sub..epsilon.Rx molecule-immobilized substrate; and binding said putative IgE-containing composition on a substrate prior to performing step (a) to form a putative IgE-containing composition-bound substrate, wherein said substrate is selected from the group consisting of a non-coated substrate, a ***Fc*** .sub..epsilon.R.alpha. molecule-immobilized substrate, an antigen-immobilized substrate and an anti-IgE antibody-immobilized substrate.

45. The method of claim 25, wherein said step of detecting comprises: (a) contacting said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex with an indicator molecule that binds selectively to said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex; (b) removing substantially all of said indicator molecule that does not selectively bind to ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex; and (c) detecting said indicator molecule, wherein presence of said indicator molecule is indicative of the presence of. . .

47. The method of claim 25, said method comprising the steps of: (a) immobilizing said ***Fc*** .sub..epsilon.R.alpha. molecule on a substrate; (b) contacting said ***Fc*** .sub..epsilon.R.alpha. molecule with said putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex.

48. The method of claim 47, wherein the presence of said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex with a compound selected from the group consisting of an antigen and an

antibody that binds selectively to. . .
49. The method of claim 48, wherein said compound comprises a ***detectable*** ***marker*** .

50. The method of claim 25, said method comprising the steps of: (a) immobilizing a specific antigen on a substrate;. . . binding to said substrate; and (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with said ***Fc*** .sub..epsilon.R.alpha. molecule.

51. The method of claim 50, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .
. . . binding to said substrate; and (d) detecting the presence of said antibody:IgE complex by contacting said antibody:IgE complex with said ***Fc*** .sub..epsilon.R.alpha. molecule.

53. The method of claim 52, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .
. . . method comprising the steps of: (a) immobilizing said putative IgE-containing composition on a substrate; (b) contacting said composition with said ***Fc*** .sub..epsilon.R.alpha. molecule under conditions suitable for formation of a ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex.

55. The method of claim 54, wherein the presence of said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex with an indicator molecule selected from the group consisting of an anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody, an antigen and a lectin.

56. The method of claim 54, wherein said ***Fc*** .sub..epsilon.R.alpha. molecule comprises a ***detectable*** ***marker*** .

58. The method of claim 25, said method comprising the steps of: (a) contacting a recombinant cell with said putative IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, wherein said recombinant cell comprises an equine ***Fc*** .sub..epsilon.R.alpha. molecule; and (b) determining the presence of IgE by detecting said recombinant cell:IgE complex, the presence of said recombinant cell:IgE. . .
59. A kit for detecting IgE comprising an equine ***Fc*** .sub..epsilon.R.alpha. protein and a means for detecting IgE.

60. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is encoded by a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule comprising a. . .
63. The kit of claim 59, wherein said detection means detects said ***Fc*** .sub..epsilon.R.alpha. protein.

64. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is connected to a ***detectable*** ***marker*** by chemical conjugation or recombinant DNA technology.

65. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is conjugated to biotin.

66. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is on the surface of a recombinant cell that comprises said ***Fc*** .sub..epsilon.R.alpha. protein.

70. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein comprises a protein selected from the group consisting of PequFc.sub..epsilon.R.alpha..sub.255, PequFc.sub..epsilon.R.alpha..sub.236 and PequFc.sub..epsilon.R.alpha..sub.201.

71. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is encoded by a nucleic acid molecule selected from the group consisting of neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 and neqFc.sub..epsilon.R.alpha..sub.603.

72. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is conjugated to a ***detectable*** ***marker*** .

73. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, an enzyme, a fluorescent label, a chemiluminescent label, a chromophoric label. . .

74. The kit of claim 59, wherein said ***Fc*** .sub..epsilon.R.alpha. protein is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

75. The kit of claim 59, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R.alpha. protein is conjugated to biotin.

. . . said labeling reagent is impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising said ***Fc*** .sub..epsilon.R.alpha. protein, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to said. . .

87. An inhibitor that interferes with formation of a complex between equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE, wherein said inhibitor is identified by its ability to interfere with said complex formation.

89. The inhibitor of claim 87, wherein said inhibitor prevents histamine release by a cell when said equine ***Fc*** .sub..epsilon.R.alpha. protein is associated with said cell.

. . . inhibitor of claim 87, wherein said inhibitor is selected from the group consisting of a substrate analog of an equine ***Fc*** .sub..epsilon.R.alpha. protein, a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein and a soluble portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein.

92. A method to identify a compound that interferes with formation of a complex between equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE, said method comprising: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of said compound, said equine ***Fc*** .sub..epsilon.R.alpha. protein forms a complex with IgE; and (b) determining if said putative inhibitory compound inhibits said complex formation.

93. A test kit to identify a compound capable of interfering with formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE, said test kit comprising an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein that can complex with IgE and a means for determining the extent of interference of said complex formation in. . .

94. A therapeutic composition that, when administered to an animal, reduces ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses, said therapeutic composition comprising a therapeutic compound selected from the group consisting of: an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein; a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R.alpha. gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. protein; and an inhibitor that interferes with

formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE.

95. The composition of claim 94, wherein said equine ***Fc*** .sub..epsilon.R.alpha. protein is selected from the group consisting of: a peptide of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE; and a soluble portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE.

. . . composition of claim 94, wherein said inhibitor is selected from the group consisting of: a substrate analog of an equine ***Fc*** .sub..epsilon.R.alpha. protein; a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein; and a soluble portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE.

98. A method to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in an animal comprising administering to an animal a therapeutic composition comprising a therapeutic compound selected from the group consisting of: an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein; a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R.alpha. gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. protein; and an inhibitor that interferes with formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE.

99. The method of claim 98, wherein said equine ***Fc*** .sub..epsilon.R.alpha. protein is selected from the group consisting of: a peptide of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE; and a soluble portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE.

. . . method of claim 98, wherein said inhibitor is selected from the group consisting of: a substrate analog of an equine ***Fc*** .sub..epsilon.R.alpha. protein; a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein; and a soluble portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE.

L13 ANSWER 6 OF 24 USPATFULL on STN
AN 2003:307129 USPATFULL
TI Feline immunoglobulin E molecules and related methods
IN McCall, Catherine, Boulder, CO, UNITED STATES
Weber, Eric, Fort Collins, CO, UNITED STATES
PI US 2003216565 A1 20031120
AI US 2003-409772 A1 20030407 (10)
RLI Division of Ser. No. US 2000-479614, filed on 7 Jan 2000, GRANTED, Pat. No. US 6573372
PRAI US 1999-115033P 19990107 (60)
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 3360
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to: nucleic acid molecules encoding the light chain and heavy chain of feline immunoglobulin E (IgE), including species-specific regions of feline IgE; proteins encoded by the nucleic acid molecules; inhibitors to the nucleic acids and proteins; antibodies to the proteins; cells transformed with the nucleic acid molecules; assays employing the transformed cells, nucleic acids, antibodies and/or proteins or portions thereof; methods for treating IgE-mediated responses (ie. allergy) using the materials provided; methods for eliciting an immune response to IgE and kits containing the nucleic acid molecules, proteins or derivatives thereof (ie. antibodies).
SUMM [0002] Allergic responses in mammals are known to be mediated by immunoglobulin E. IgE molecules bind the ***Fc*** . ***epsilon*** .

receptor on mast cells and, when complexed with antigen, trigger a cascade of events that leads to the release of allergic mediators (ie. histamine, prostaglandins and proteases). Thus, interference with the IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction is an avenue for controlling allergic responses. Interference with the IgE antibody/ ***Fc*** . ***epsilon*** . ***receptor*** interaction will also affect the pathology of atopic disease, hyper IgE syndrome, internal parasite infections and B cell neoplasia.

SUMM [0003] The species-specific portion of the IgE, the IgE constant region (on the heavy chain and involved in ***Fc*** . ***epsilon*** . ***receptor*** binding) is of particular importance in design and manufacture of compounds useful to interfere with the IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction, because compounds which are specific for this region produce little interference with non-IgE/receptor interactions. Moreover, the IgE constant region. . .

SUMM [0004] The DNA and amino acid sequences of IgE molecules from several species, including ***human*** , rat, mouse and dog, have been reported. Peptides derived from known IgE sequences have been used to generate antibodies which. . . IgE-mediated allergic symptoms through the use of monoclonal or polyclonal antibodies raised against epitopes present in B cell-associated or soluble ***human*** IgE. WO90/15878 discloses the use of peptides derived from ***human*** , rat or mouse IgE sequences to generate antibodies which inhibit IgE-mediated mast cell degranulation. U.S. Pat. No. 4,223,016 discloses the. . .

SUMM . . . the time of filing, nucleic acid molecules which encode those which encode the constant region, specifically those which encode a ***Fc*** . ***epsilon*** . ***receptor*** (sometimes called " ***Fc*** .epsilon.R") binding region, is preferred. In particular, nucleic acid molecules which encode a feline IgE ***Fc*** . ***epsilon*** . ***receptor*** binding region and which comprises SEQ ID NO 4, SEQ ID NO 7 or SEQ ID NO 10 are preferred.. . .

SUMM [0020] The preferred embodiments of this aspect of the present invention include those proteins capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** , in particular, SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11 and SEQ ID NO 14.

SUMM [0028] Also provided by the present invention are methods to identify the ability of a test compound to interfere with IgE/ ***Fc*** .epsilon. interaction, comprising: contacting the test compound with a protein of the present invention; and determining whether the test compound and. . .

SUMM [0048] " ***Fc*** . ***epsilon*** . ***receptor*** " means any ***Fc*** . ***epsilon*** . ***receptor*** recognized in the art, including the "low" affinity or "high" affinity receptors, or any such new receptors discovered.

SUMM [0049] "Feline ***Fc*** . ***epsilon*** . ***receptor*** binding region" means a region of the feline IgE molecule that is capable of binding to a ***Fc*** . ***epsilon*** . ***receptor*** , including the entire, naturally-occurring binding region, portions thereof that bind to the ***Fc*** . ***epsilon*** . ***receptor*** , or modifications of either the entire naturally-occurring binding region or portions thereof.

SUMM . . . IgE-mediated immune response" means not only any humoral or cellular immune response, but also any biological response resulting from an IgE/ ***Fc*** . ***epsilon*** . ***receptor***. interaction.

SUMM . . . At the time of filing, nucleic acids which encode the constant region, specifically those nucleic acid molecules which encode the ***Fc*** . ***epsilon*** . ***receptor*** binding region, are preferred. In particular, SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, and SEQ. . .

SUMM . . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit feline ***Fc*** .epsilon.R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the. . .

SUMM . . . of a portion of a IgE heavy chain 2

reverse DNA complement to 1 3

DNA sequence which encodes the most preferred ***Fc*** .epsilon.R binding 4 region of the IgE heavy chain

AA sequence which is the most preferred ***Fc*** .epsilon.R binding region of 5

the IgE heavy chain
reverse DNA complement to 3 6
DNA sequence of more preferred ***Fc*** .epsilon.R binding region 7
AA sequence of 7 8
reverse DNA complement to 7 9
DNA sequence of preferred ***Fc*** .epsilon.R binding region 10
AA sequence of 10 11
reverse DNA complement to 8 12
DNA sequence of constant region 13
AA sequence of constant. . .

SUMM . . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM . . . feline IgE mimetope; a feline IgE substrate analog; or a feline IgE peptide. Preferably, a feline IgE molecule binds to ***Fc*** . ***epsilon*** . ***receptors*** .

SUMM . . . compositions comprising the two, as well as portions of either. In particular, isolated feline constant region proteins are preferred, although ***Fc*** .epsilon. binding region proteins are most preferred. Proteins which would result from expression of the nucleic acid molecules herein disclosed are. . .

SUMM . . . the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to ***Fc*** . ***epsilon*** . ***receptor*** . Examples of feline IgE protein homologs include feline IgE proteins in which amino acids have been deleted (e.g., a truncated. . . phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog is capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** .

SUMM . . . any compound that is able to mimic the activity of such a feline IgE protein (e.g., ability to bind to ***Fc*** . ***epsilon*** . ***receptors***), often because the mimetope has a structure that mimics the feline IgE protein. It is to be noted, however, that. . . or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline ***Fc*** . ***epsilon*** . ***receptor*** domain or anti-feline IgE antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational. . . peptidomimetic compound that is structurally and/or functionally similar to a feline IgE protein of the present invention, particularly to the ***Fc*** . ***epsilon*** . ***receptor*** -binding domain of the feline IgE protein.

SUMM . . . metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . .

SUMM . . . the present invention can also include chimeric molecules comprising a portion of a feline IgE molecule that binds to an ***Fc*** . ***epsilon*** . ***receptor*** and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the IgE molecule portion binds to ***Fc*** .epsilon.R in essentially the same manner as an IgE molecule that is not bound to a substrate. An example of a. . .

SUMM . . . antibodies selective for the constant region of the feline IgE heavy chain, although more preferred are antibodies selective for the ***Fc*** . ***epsilon*** . ***receptor*** -binding domain of the IgE heavy chain. In one preferred embodiment, there are provided antibodies selective for a protein selected from. . .

SUMM . . . invention. For example, such antibodies can be used (a) as tools to detect IgE in the presence or absence of ***Fc*** . ***epsilon*** . ***receptor*** and/or (b) as tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture. . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells having ***Fc*** . ***epsilon*** . ***receptors*** in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably joining) such antibodies to the. . . in the art. Suitable cytotoxic agents are known to those

skilled in the art. Antibodies of the present invention, including ***Fc*** . ***epsilon*** . ***receptor*** binding site-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** . ***epsilon*** . ***receptors*** , to produce anti-feline IgE idiotypic antibodies, to purify cells having feline IgE proteins, to stimulate intracellular signal transduction through a feline ***Fc*** .epsilon. and to identify cells having feline IgE proteins.

SUMM [0137] By "inhibitor" it is meant that the compound inhibits the formation of a complex between feline IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** . Such inhibitors can, for example, interact with the feline ***Fc*** . ***epsilon*** . ***receptor*** binding site on IgE, other regions on feline IgE that effect IgE binding to ***Fc*** . ***epsilon*** . ***receptor*** or the IgE binding site, for example, by allosteric interaction, on ***Fc*** . ***epsilon*** . ***receptor*** . An inhibitor of IgE and ***Fc*** . ***epsilon*** . ***receptor*** complex formation protein can interfere with complex formation by, for example, preventing formation of an IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** complex or disrupting an existing IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** complex causing the IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** to dissociate. An inhibitor of IgE and ***Fc*** . ***epsilon*** . ***receptor*** complex formation is usually a relatively small molecule. Preferably, an inhibitor of the present invention is derived from a feline IgE of the present invention, and more preferably from the ***Fc*** . ***epsilon*** . ***receptor*** binding site of the IgE, and is identified by its ability to bind to, or otherwise interact with, a ***Fc*** . ***epsilon*** . ***receptor*** protein, thereby interfering with the formation of a complex between a feline IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** .

SUMM . . . IgE protein of the present invention include, but are not limited to, feline IgE proteins, fragments or mimetopes thereof, a ***Fc*** . ***epsilon*** . ***receptor*** binding analog of a feline IgE protein, and other molecules that bind to a feline IgE protein (e.g., to an allosteric site) or ***Fc*** . ***epsilon*** . ***receptor*** in such a manner that ***Fc*** . ***epsilon*** . ***receptor*** and IgE protein complex formation is inhibited. Preferred feline IgE proteins, fragments and mimetopes thereof are capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** in such a manner that feline IgE does not bind to ***Fc*** . ***epsilon*** . ***receptor*** . Mimetopes include those disclosed herein.

SUMM . . . A feline IgE protein binding analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the ***Fc*** .. ***epsilon*** . ***receptor*** -binding site of a feline IgE protein. A preferred feline IgE protein binding analog inhibits ***Fc*** . ***epsilon*** . ***receptor*** -binding activity of a feline IgE protein. Feline IgE protein binding analogs can be of any inorganic or organic composition, and,. . . IgE protein substrate analogs can be, but need not be, structurally similar to a feline IgE protein's natural substrate (e.g., ***Fc*** . ***epsilon*** . ***receptor***) as long as they can interact with the active site (e.g., ***Fc*** . ***epsilon*** . ***receptor*** -binding site of that feline IgE). Feline IgE protein binding analogs can be designed using computer-generated structures of feline IgE proteins of the present invention or computer structures of, for example, the IgE-binding domain of ***Fc*** . ***epsilon*** . ***receptor*** . Binding analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic compounds, or other. . . protein binding analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural feline ***Fc*** . ***epsilon*** . ***receptor*** protein, particularly to the region of the substrate that binds to a feline IgE protein, but that inhibits IgE binding upon interacting with the ***Fc*** . ***epsilon*** . ***receptor*** binding site).

SUMM . . . manner that a therapeutic compound (e.g., an inhibitor of a feline IgE protein, an anti-feline IgE antibody, an inhibitor of ***Fc*** . ***epsilon*** . ***receptor*** , or nucleic acid molecules encoding feline IgE proteins) binds to an IgE molecule in the

animal. Such administration could be. . .

SUMM . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** . ***epsilon*** . ***receptor*** -mediated biological responses in the animal. As used herein, ***Fc*** . ***epsilon*** . ***receptor*** -mediated biological response refers to cellular responses that occur when IgE is complexed with ***Fc*** . ***epsilon*** . ***receptor*** . For example, a ***Fc*** .epsilon.-mediated biological response includes release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . of skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen-specific ***Fc*** . ***epsilon*** . ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** . ***epsilon*** . ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising a feline IgE nucleic acid molecule of the present. . .

SUMM . . . or feline IgE protein, or other modulators of feline IgE activity, such as mimetopes, analogs, homologs, chimeras which inhibit the IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction, may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier, or by. . .

SUMM . . . such a manner as to not substantially interfere with the ability of the feline IgE molecules to selectively bind to ***Fc*** . ***epsilon*** . ***receptor*** .

SUMM . . . of: (a) administering to an animal an effective amount of a therapeutic composition of an inhibitor of feline IgE and ***Fc*** . ***epsilon*** . ***receptor*** complex formation. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According to the present invention, a therapeutic. .

.

SUMM [0168] The present invention also provides methods to identify the ability of a test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction, comprising: contacting the test compound with a protein of the present invention; and determining whether the test compound and. . .

SUMM [0169] In particular, there are provided methods to identify the ability of a test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction comprising: (a) contacting an isolated feline IgE molecule with a test compound/ ***Fc*** . ***epsilon*** . ***receptor*** containing solution under conditions suitable for formation of an IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex; and (b) determining the ability of the test compound to interfere with IgE/ ***Fc*** .epsilon. interaction by detecting the IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex, the presence of the IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex indicating the presence of IgE. A preferred feline IgE molecule is one which a carbohydrate group of the feline. . .

SUMM . . . Another embodiment of the present invention is a method to identify the ability of a test compound to interfere with IgE/ ***Fc*** .epsilon. interaction comprising: (a) contacting a ***Fc*** . ***epsilon*** . ***receptor*** -bearing cell test compound and an IgE molecule of the present invention under conditions suitable for formation of a recombinant cell:IgE complex; and (b) determining the ability of the test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the ability of the test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction.

SUMM [0171] A preferred method to detect the ability of the test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction comprises: (a) immobilizing a presently-disclosed IgE or a

Fc . ***epsilon*** . ***receptor*** molecule on a substrate; (b) contacting the IgE or ***Fc*** . ***epsilon*** . ***receptor*** molecule with the test compound under conditions suitable for formation of an IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** . ***epsilon*** . ***receptor*** molecule:IgE complex binding to the substrate; and (d) detecting the presence of the IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex.

SUMM [0173] A preferred method to detect IgE comprises: (a) immobilizing ***Fc*** . ***epsilon*** . ***receptor*** on a substrate; (b) contacting a test compound with a presently-disclosed feline IgE molecule under conditions suitable for formation of a IgE:test compound complex bound to the ***Fc*** . ***epsilon*** . ***receptor*** on the substrate; (c) removing non-bound material from the ***Fc*** . ***epsilon*** . ***receptor*** on the substrate under conditions that retain feline IgE molecule:test compound complex binding to the ***Fc*** . ***epsilon*** . ***receptor*** on the substrate; and (d) detecting the presence of the feline IgE molecule:test compound complex.

SUMM . . . IgE. Preferred IgE to detect using a feline IgE nucleic acid molecule include feline IgE, canine IgE, equine IgE and ***human*** IgE, with feline IgE being particularly preferred.

SUMM . . . the ability of the molecules to form a stable complex that can be measured (i.e., detected). Binding between a feline ***Fc*** . ***epsilon*** . ***receptor*** and a feline IgE molecule is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, e.g., between ***Fc*** . ***epsilon*** . ***receptor*** and feline IgE molecules in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the feline IgE molecule or to a reagent that selectively binds to the feline IgE protein or nucleic acid. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable*** ***marker*** can be connected to a feline IgE molecule using, for example, chemical conjugation or recombinant DNA technology (e.g., connection of. . .

SUMM . . . one embodiment, a complex is detected by contacting a test compound with a feline IgE that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a feline IgE molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to a feline IgE molecule in such a manner as not to block the ability of the feline. . .

SUMM . . . a feline IgE molecule (referred to herein as an anti-feline IgE antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a feline IgE molecule, such as ***human*** ***Fc*** . ***epsilon*** . ***receptor*** , Feline ***Fc*** . ***epsilon*** . ***receptor*** , or an antigen that binds to an IgE. A feline IgE molecule conjugated to biotin is preferably detected using streptavidin,. . . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** . Another preferred method is a flow-through assay, examples of which are disclosed in U.S. Pat. No. 4,727,019, issued Feb. 23,. . .

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary

antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

SUMM . . . complex between the indicator molecule and the feline IgE molecule:test compound complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a calorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . added if required, and the substrate is submitted to a detection device for analysis. Preferred test compounds to detect are ***Fc*** .epsilon.R from any animal, antigens or anti-IgE antibodies.

SUMM . . . a complex between the feline IgE molecule and the test compound. Preferably, the feline IgE molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline IgE molecule is removed, a developing agent is. . . added if required, and the substrate is submitted to a detection device for analysis. Preferred test compounds to detect are ***Fc*** . ***epsilon*** . ***receptor*** from any animal, antigens or antiIgE antibodies.

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . .

SUMM . . . constant region is preferred. More preferred is a method as above, wherein the portion of the IgE molecule is the ***Fc*** . ***epsilon*** . ***receptor*** -binding region. Most preferred is a method as above wherein the portion of the IgE molecule is SEQ ID NO 2,. . .

SUMM . . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a feline IgE protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

SUMM . . . kit of the present invention are useful for diagnosing abnormal conditions in animals that are associated with changing levels of ***Fc*** . ***epsilon*** . ***receptor*** . Particularly preferred conditions to diagnose include allergies, parasitic infections and neoplasia. For example, a method and kit of the present. . .

DETD . . . feline IgE and do not bind to IgG or IgM. These five antibodies also do not react with canine or ***human*** IgE. In addition, three monoclonal antibodies reactive to feline IgE light chain were produced and are referred to as H-99,. . .

CLM What is claimed:

2. An isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a feline IgE ***Fc*** . ***epsilon*** . ***receptor*** binding region and which comprises SEQ ID NO 4.

3. An isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a feline IgE ***Fc*** . ***epsilon*** . ***receptor*** binding region and which comprises SEQ ID NO 7.

4. An isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a feline IgE ***Fc*** . ***epsilon*** . ***receptor*** binding region and which comprises SEQ ID NO 10.

9. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** and comprises SEQ ID NO 11.

10. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** and comprises SEQ ID NO 8.

11. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** and comprises SEQ ID NO 5.

12. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** and

comprises SEQ ID NO 14.

17. A method to identify the ability of a test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction, comprising: contacting the test compound with a protein of claim 8; and determining whether the test compound and said. . .

L13 ANSWER 7 OF 24 USPATFULL on STN
AN 2003:140937 USPATFULL
TI NOVEL DERMATOPHAGOIDES PROTEINS AND USES THEREOF
IN McCall, Catherine A., Boulder, CO, UNITED STATES
Hunter, Shirley Wu, Fort Collins, CO, UNITED STATES
Weber, Eric R., Fort Collins, CO, UNITED STATES
PI US 2003096779 A1 20030522
AI US 2002-218743 A1 20020813 (10)
RLI Division of Ser. No. US 1999-292225, filed on 15 Apr 1999, GRANTED, Pat. No. US 6455686
PRAI US 1998-98909P 19980902 (60)
US 1998-85295P 19980513 (60)
US 1998-98565P 19980417 (60)
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 5292
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to high molecular weight Dermatophagoides proteins, nucleic acid molecules encoding such proteins, and therapeutic and diagnostic reagents derived from such proteins.
SUMM . . . of the present invention, nor the relevance of such proteins as being immunoreactive with IgE antibodies in canine, feline, or ***human*** sera.
DETD . . . metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . .
DETD . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the. . .
DETD . . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .
DETD . . . Particularly preferred animals to test with a skin test of the present invention include humans, canines, felines and equines, with ***human*** , canines and felines being even more preferred. As used herein, canine refers to any member of the dog family, including. . .
DETD . . . having an IgE or IgG isotype. Preferred anti-Der HMW-map antibody to detect include feline antibody, canine antibody, equine antibody and ***human*** antibody, with feline, canine and ***human*** antibody being particularly preferred.
DETD . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** .
DETD [0116] In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the Der HMW-map protein, to antibody bound to the Der HMW-map protein, or to a reagent that selectively binds. . .
DETD . . . a complex is detected by contacting a putative antibody-containing composition with a Der HMW-map protein that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a Der HMW-map protein includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker***

is conjugated to a Der HMW-map protein in such a manner as not to block the ability of the Der. . .

DETD . . . composition. Preferred lectins include those lectins that bind to high-mannose groups. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

DETD . . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. Preferred indicator molecules include, but are not limited to, an anti-feline IgE antibody, an anti-feline IgG antibody, an anti-canine IgE antibody, an anti-canine IgG antibody, an anti- ***human*** IgE antibody, and an anti- ***human*** IgG antibody. As used herein, an anti-IgE or anti-IgG antibody includes not only a complete antibody but also any subunit. . .

DETD . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

DETD . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

DETD . . . HMW-map protein and the anti-IgE antibody:IgE complex or anti-IgG antibody:IgG complex. Preferably, the Der HMW-map protein is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess Der HMW-map protein is removed, a developing agent is. . .

DETD . . . complex between the Der HMW-map protein and the IgE or IgG. Preferably, the Der HMW-map protein is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess Der HMW-map protein is removed, a developing agent is. . .

DETD . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . .

DETD . . . antibody capable of selectively binding to an IgE or IgG disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a Der HMW-map protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin).

DETD . . . compound include variable regions capable of binding to immune cell specific surface molecules and constant regions capable of binding to ***Fc*** receptors on immune cells, in particular IgE constant regions. Preferred CD8 molecules include at least the extracellular functional domains of. . .

DETD . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

DETD . . . that occur when mite allergens contact an animal. For example, IgE that specifically binds to mite allergen becomes coupled with ***Fc*** ***epsilon*** ***receptor*** , resulting in ***Fc*** ***epsilon*** ***receptor*** -mediated biological response including release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

DETD . . . regulating adenosine 3',5'-cyclic phosphate (cAMP) activity, and compounds that block IgE activity, such as peptides from IgE or IgE specific ***Fc*** receptors, antibodies specific for peptides from IgE or IgE-specific ***Fc*** receptors, or antibodies capable of blocking binding of IgE to ***Fc*** receptors.

DETD . . . room temperature and then washed four times with PBST. About 100 .mu.l/well of a 1:4000 dilution of 40 .mu.g/ml biotinylated ***human*** ***Fc*** .epsilon.R alpha chain protein (produced as

described in Frank et al., WO 98/23964, published Nov. 24, 1997) contained in PBSTFCS was. . .

DETD . . . determined. The plate was incubated for about 1 hour at room temperature and then washed four times with PBST. Biotinylated ***human*** ***Fc*** .epsilon.R alpha chain protein was then added and the presence of IgE bound to the plate was detected using the methods. . .

DETD . . . determined. The plate was incubated for about 1 hour at room temperature and then washed four times with PBST. Biotinylated ***human*** ***Fc*** .epsilon.R alpha chain protein was then added and the presence of IgE bound to the plate was detected using the methods. . .

DETD [0234] This example demonstrates the binding of the D. farinae HMW-map composition to ***human*** IgE in ***human*** sera isolated from humans known to be allergic to mite allergens.

DETD [0235] A technique called RAST, or radio-allergo-absorbent test, was used because the amount of ***human*** IgE present in ***human*** sera is quite low. RAST was essentially performed as described in Aalberse, R C et al., (1981) J. Allergy Clin. . . To calculate the unit IU/ml, a standard curve was derived by performing RAST with several dilutions of a well-characterized chimeric ***human*** /mouse IgE monoclonal antibody against Derp2, (***human*** IgE/monoclonal anti-Derp2, following the procedure of Schuurman, et al. (1997) J Allergy Clin Immunol. 99: pp 545-550).

DETD . . . 1, was coupled to 50 mg of CNBr-activated Sepharose 4B (available from Pharmacia, Piscataway, N.J.), according to the manufacturer's protocols. ***Human*** sera were selected (17 different samples, total) on the basis of a positive RAST for whole mite D. farinae extracts,. . .

DETD . . . overnight at 27.degree. C., with shaking. After incubation, the coupled Sepharose was washed five times with PBS-T. Radiolabelled (.sup.125-Iodine) sheep anti- ***human*** IgE, made by standard radioiodination protocols, (diluted in PBS-T with 4.5% bovine serum and 0.5% sheep serum, v/v) in a. . . coupled Sepharose was washed four times with PBS-T and counted in a gamma-counter to determine the amount of radiolabeled sheep anti- ***human*** IgE bound to the HMW-map composition-coupled Sepharose. The results are shown in Table 4.

TABLE 4

Binding of ***human*** IgE to HMW-map composition from D. farinae

Serum number	RAST, D. farinae whole extract, IU	RAST, HMW-map comps'n., IU
1445	>100	48
1456. . .		

L13 ANSWER 8 OF 24 USPATFULL on STN
AN 2003:17431 USPATFULL
TI FELINE IMMUNOGLOBULIN E MOLECULES AND RELATED METHODS
IN MCCALL, CATHERINE, BOULDER, CO, UNITED STATES
WEBER, ERIC, FORT COLLINS, CO, UNITED STATES
PI US 2003013183 A1 20030116
US 6573372 B2 20030603
AI US 2000-479614 A1 20000107 (9)
PRAI US 1999-115033P 19990107 (60)
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 3368
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to: nucleic acid molecules encoding the light chain and heavy chain of feline immunoglobulin E (IgE), including species-specific regions of feline IgE; proteins encoded by the nucleic acid molecules; inhibitors to the nucleic acids and proteins; antibodies to the proteins; cells transformed with the nucleic acid molecules; assays employing the transformed cells, nucleic acids, antibodies and/or

proteins or portions thereof; methods for treating IgE-mediated responses (ie. allergy) using the materials provided; methods for eliciting an immune response to IgE and kits containing the nucleic acid molecules, proteins or derivatives thereof (ie. antibodies).

SUMM [0002] Allergic responses in mammals are known to be mediated by immunoglobulin E. IgE molecules bind the ***Fc*** . ***epsilon*** . ***receptor*** on mast cells and, when complexed with antigen, trigger a cascade of events that leads to the release of allergic mediators (ie. histamine, prostaglandins and proteases). Thus, interference with the IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction is an avenue for controlling allergic responses. Interference with the IgE antibody/ ***Fc*** . ***epsilon*** . ***receptor*** interaction will also affect the pathology of atopic disease, hyper IgE syndrome, internal parasite infections and B cell neoplasia.

SUMM [0003] The species-specific portion of the IgE, the IgE constant region (on the heavy chain and involved in ***Fc*** . ***epsilon*** . ***receptor*** binding) is of particular importance in design and manufacture of compounds useful to interfere with the IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction, because compounds which are specific for this region produce little interference with non-IgE/receptor interactions. Moreover, the IgE constant region. . .

SUMM [0004] The DNA and amino acid sequences of IgE molecules from several species, including ***human*** , rat, mouse and dog, have been reported. Peptides derived from known IgE sequences have been used to generate antibodies which. . . IgE-mediated allergic symptoms through the use of monoclonal or polyclonal antibodies raised against epitopes present in B cell-associated or soluble ***human*** IgE. WO90/15878 discloses the use of peptides derived from ***human*** , rat or mouse IgE sequences to generate antibodies which inhibit IgE-mediated mast cell degranulation. U.S. Pat. No. 4,223,016 discloses the. . .

SUMM . . . the time of filing, nucleic acid molecules which encode those which encode the constant region, specifically those which encode a ***Fc*** . ***epsilon*** . ***receptor*** (sometimes called " ***Fc*** .epsilon.R") binding region, is preferred. In particular, nucleic acid molecules which encode a feline IgE ***Fc*** . ***epsilon*** . ***receptor*** binding region and which comprises SEQ ID NO 4, SEQ ID NO 7 or SEQ ID NO 10 are preferred.. . .

SUMM [0020] The preferred embodiments of this aspect of the present invention include those proteins capable of binding to ***Fc*** receptor, in particular, SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11 and SEQ ID NO 14.

SUMM [0028] Also provided by the present invention are methods to identify the ability of a test compound to interfere with IgE/ ***Fc*** .epsilon., interaction, comprising: contacting the test compound with a protein of the present invention; and determining whether the test compound and. . .

SUMM [0048] " ***Fc*** . ***epsilon*** . ***receptor*** " means any ***Fc*** . ***epsilon*** . ***receptor*** recognized in the art, including the "low" affinity or "high" affinity receptors, or any such new receptors discovered.

SUMM [0049] "Feline ***Fc*** . ***epsilon*** . ***receptor*** binding region" means a region of the feline IgE molecule that is capable of binding to a ***Fc*** . ***epsilon*** . ***receptor*** , including the entire, naturally-occurring binding region, portions thereof that bind to the ***Fc*** . ***epsilon*** . ***receptor*** , or modifications of either the entire naturally-occurring binding region or portions thereof.

SUMM . . . IgE-mediated immune response" means not only any humoral or cellular immune response, but also any biological response resulting from an IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction.

SUMM . . . At the time of filing, nucleic acids which encode the constant region, specifically those nucleic acid molecules which encode the ***Fc*** . ***epsilon*** . ***receptor*** binding region, are preferred. In particular, SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, and SEQ. . .

SUMM . . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit feline ***Fc*** .epsilon.R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the. . .

SUMM . . . of a portion of a IgE heavy chain

2
reverse DNA complement to 1
3
DNA sequence which encodes the most preferred ***Fc*** .epsilon.R binding region of the IgE heavy chain 4
AA sequence which is the most preferred ***Fc*** .epsilon.R binding region of the IgE heavy chain 5
reverse DNA complement to 3
6
DNA sequence of more preferred ***Fc*** .epsilon.R binding region
7
AA sequence of 7
8
reverse DNA complement to 7
9
DNA sequence of preferred ***Fc*** .epsilon.R binding region
10
AA sequence of 10
11
reverse DNA complement to 8
12
DNA sequence of constant region
13
AA sequence of constant. . .

SUMM . . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM . . . feline IgE mimetope; a feline IgE substrate analog; or a feline IgE peptide. Preferably, a feline IgE molecule binds to ***Fc*** . ***epsilon*** . ***receptors*** .

SUMM . . . compositions comprising the two, as well as portions of either. In particular, isolated feline constant region proteins are preferred, although ***Fc*** .epsilon. binding region proteins are most preferred. Proteins which would result from expression of the nucleic acid molecules herein disclosed are. . .

SUMM . . . the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to ***FcpE*** receptor. Examples of feline IgE protein homologs include feline IgE proteins in which amino acids have been deleted (e.g., a. . . phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog is capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** .

SUMM . . . any compound that is able to mimic the activity of such a feline IgE protein (e.g., ability to bind to ***Fc*** . ***epsilon*** . ***receptors***), often because the mimetope has a structure that mimics the feline IgE protein. It is to be noted, however, that. . . or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline ***Fc*** . ***epsilon*** . ***receptor*** domain or anti-feline IgE antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational. . . peptidomimetic compound that is structurally and/or functionally similar to a feline IgE protein of the present invention, particularly to the ***Fc*** . ***epsilon*** . ***receptor*** -binding domain of the feline IgE protein.

SUMM . . . metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . .

SUMM . . . the present invention can also include chimeric molecules comprising a portion of a feline IgE molecule that binds to an ***Fc*** . ***epsilon*** . ***receptor*** and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the IgE molecule portion binds to ***Fc*** .epsilon.R in essentially the same manner as an IgE molecule that is not bound to a substrate. An example of a. . .

SUMM . . . antibodies selective for the constant region of the feline IgE

heavy chain, although more preferred are antibodies selective for the ***Fc*** . ***epsilon*** . ***receptor*** -binding domain of the IgE heavy chain. In one preferred embodiment, there are provided antibodies selective for a protein selected from. . .

SUMM . . . invention. For example, such antibodies can be used (a) as tools to detect IgE in the presence or absence of ***Fc*** . ***epsilon*** . ***receptor*** and/or (b) as tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture. . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells having ***Fc*** . ***epsilon*** . ***receptors*** in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably joining) such antibodies to the. . . in the art. Suitable cytotoxic agents are known to those skilled in the art. Antibodies of the present invention, including ***Fc*** . ***epsilon*** . ***receptor*** binding site-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** . ***epsilon*** . ***receptors*** , to produce anti-feline IgE idiotypic antibodies, to purify cells having feline IgE proteins, to stimulate intracellular signal transduction through a feline ***Fc*** .epsilon. and to identify cells having feline IgE proteins.

SUMM [0137] By "inhibitor" it is meant that the compound inhibits the formation of a complex between feline IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** . Such inhibitors can, for example, interact with the feline ***Fc*** . ***epsilon*** . ***receptor*** binding site on IgE, other regions on feline IgE that effect IgE binding to ***Fc*** . ***epsilon*** ., ***receptor*** or the IgE binding site, for example, by allosteric interaction, on ***Fc*** . ***epsilon*** . ***receptor*** . An inhibitor of IgE and ***Fc*** . ***epsilon*** . ***receptor*** complex formation protein can interfere with complex formation by, for example, preventing formation of an IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** complex or disrupting an existing IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** complex causing the IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** to dissociate. An inhibitor of IgE and ***Fc*** . ***epsilon*** . ***receptor*** complex formation is usually a relatively small molecule. Preferably, an inhibitor of the present invention is derived from a feline IgE of the present invention, and more preferably from the ***Fc*** . ***epsilon*** . ***receptor*** binding site of the IgE, and is identified by its ability to bind to, or otherwise interact with, a ***Fc*** . ***epsilon*** . ***receptor*** protein, thereby interfering with the formation of a complex between a feline IgE protein and ***Fc*** . ***epsilon*** . ***receptor*** .

SUMM . . . IgE protein of the present invention include, but are not limited to, feline IgE proteins, fragments or mimetopes thereof, a ***Fc*** . ***epsilon*** . ***receptor*** binding analog of a feline IgE protein, and other molecules that bind to a feline IgE protein (e.g., to an allosteric site) or ***Fc*** . ***epsilon*** . ***receptor*** in such a manner that ***Fc*** . ***epsilon*** . ***receptor*** and IgE protein complex formation is inhibited. Preferred feline IgE proteins, fragments and mimetopes thereof are capable of binding to ***Fc*** . ***epsilon*** . ***receptor*** in such a manner that feline IgE does not bind to ***Fc*** . ***epsilon*** . ***receptor*** . Mimetopes include those disclosed herein.

SUMM . . . A feline IgE protein binding analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the ***Fc*** . ***epsilon*** . ***receptor*** -binding site of a feline lgE protein. A preferred feline IgE protein binding analog inhibits ***Fc*** . ***epsilon*** . ***receptor*** -binding activity of a feline IgE protein. Feline IgE protein binding analogs can be of any inorganic or organic composition, and,. . . IgE protein substrate analogs can be, but need not be, structurally similar to a feline IgE protein's natural substrate (e.g., ***Fc*** . ***epsilon*** . ***receptor***) as long as they can interact with the active site (e.g., ***Fc*** . ***epsilon*** . ***receptor*** -binding site of that feline IgE). Feline IgE protein binding analogs can be designed using computer-generated structures of feline IgE proteins of the present invention or computer structures of, for

example, the IgE-binding domain of ***Fc*** . ***epsilon*** . ***receptor*** . Binding analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic compounds, or other. . . protein binding analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural feline ***Fc*** . ***epsilon*** ., ***receptor*** protein, particularly to the region of the substrate that binds to a feline IgE protein, but that inhibits IgE binding upon interacting with the ***Fc*** . ***epsilon*** . ***receptor*** binding site).

SUMM . . . manner that a therapeutic compound (e.g., an inhibitor of a feline IgE protein, an anti-feline IgE antibody, an inhibitor of ***Fc*** . ***epsilon*** . ***receptor*** , or nucleic acid molecules encoding feline IgE proteins) binds to an IgE molecule in the animal. Such administration could be. . .

SUMM . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** . ***epsilon*** ., ***receptor*** -mediated biological responses in the animal. As used herein, ***Fc*** . ***epsilon*** . ***receptor*** -mediated biological response refers to cellular responses that occur when IgE is complexed with ***Fc*** . ***epsilon*** . ***receptor*** . For example, a ***Fc*** .epsilon.-mediated biological response includes release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . of skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen-specific ***Fc*** . ***epsilon*** . ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** . ***epsilon*** . ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising a feline IgE nucleic acid molecule of the present. . .

SUMM . . . or feline IgE protein, or other modulators of feline IgE activity, such as mimetopes, analogs, homologs, chimeras which inhibit the IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction, may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier, or by. . .

SUMM . . . such a manner as to not substantially interfere with the ability of the feline IgE molecules to selectively bind to ***Fc*** . ***epsilon*** . ***receptor*** .

SUMM . . . of: (a) administering to an animal an effective amount of a therapeutic composition of an inhibitor of feline IgE and ***Fc*** . ***epsilon*** . ***receptor*** complex formation. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According to the present invention, a therapeutic. .

SUMM [0168] The present invention also provides methods to identify the ability of a test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction, comprising: contacting the test compound with a protein of the present invention; and determining whether the test compound and. . .

SUMM [0169] In particular, there are provided methods to identify the ability of a test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction comprising: (a) contacting an isolated feline IgE molecule with a test compound/ ***Fc*** . ***epsilon*** . ***receptor*** containing solution under conditions suitable for formation of an IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex; and (b) determining the ability of the test compound to interfere with IgE/ ***Fc*** .epsilon. interaction by detecting the IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex, the presence of the IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex indicating the presence of IgE. A preferred feline IgE molecule is one which a carbohydrate group of the feline. . .

SUMM . . . Another embodiment of the present invention is a method to identify the ability of a test compound to interfere with IgE/ ***Fc*** .epsilon. interaction comprising: (a) contacting a ***Fc*** . ***epsilon*** . ***receptor*** -bearing cell test compound and an IgE molecule of the present invention under conditions suitable for formation of a recombinant cell:IgE complex; and (b) determining the ability of the test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the ability of the test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction.

SUMM [0171] A preferred method to detect the ability of the test compound to interfere with IgE/ ***Fc*** . ***epsilon*** . ***receptor*** interaction comprises: (a) immobilizing a presently-disclosed IgE or a ***Fc*** . ***epsilon*** . ***receptor*** molecule on a substrate; (b) contacting the IgE or ***Fc*** . ***epsilon*** . ***receptor*** molecule with the test compound under conditions suitable for formation of an IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** . ***epsilon*** . ***receptor*** molecule:IgE complex binding to the substrate; and (d) detecting the presence of the IgE molecule: ***Fc*** . ***epsilon*** . ***receptor*** complex.

SUMM [0173] A preferred method to detect IgE comprises: (a) immobilizing ***Fc*** . ***epsilon*** . ***receptor*** on a substrate; (b) contacting a test compound with a presently-disclosed feline IgE molecule under conditions suitable for formation of a IgE:test compound complex bound to the ***Fc*** . ***epsilon*** . ***receptor*** on the substrate; (c) removing non-bound material from the ***Fc*** . ***epsilon*** . ***receptor*** on the substrate under conditions that retain feline IgE molecule:test compound complex binding to the ***Fc*** . ***epsilon*** . ***receptor*** on the substrate; and (d) detecting the presence of the feline IgE molecule:test compound complex.

SUMM . . . IgE. Preferred IgE to detect using a feline IgE nucleic acid molecule include feline IgE, canine IgE, equine IgE and ***human*** IgE, with feline IgE being particularly preferred.

SUMM . . . the ability of the molecules to form a stable complex that can be measured (i.e., detected). Binding between a feline ***Fc*** . ***epsilon*** . ***receptor*** and a feline IgE molecule is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, e.g., between ***Fc*** . ***epsilon*** . ***receptor*** and feline IgE molecules in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the feline IgE molecule or to a reagent that selectively binds to the feline IgE protein or nucleic acid. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable*** ***marker*** can be connected to a feline IgE molecule using, for example, chemical conjugation or recombinant DNA technology (e.g., connection of. . .

SUMM . . . one embodiment, a complex is detected by contacting a test compound with a feline IgE that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a feline IgE molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to a feline IgE molecule in such a manner as not to block the ability of the feline. .

SUMM . . . a feline IgE molecule (referred to herein as an anti-feline IgE antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a feline IgE molecule, such as ***human*** ***Fc*** . ***epsilon*** . ***receptor*** , Feline

Fc . ***epsilon*** . ***receptor*** , or an antigen that binds to an IgE. A feline IgE molecule conjugated to biotin is preferably detected using streptavidin,. . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** . Another preferred method is a flow-through assay, examples of which are disclosed in U.S. Pat. No. 4,727,019, issued Feb. 23,. . .

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

SUMM . . . complex between the indicator molecule and the feline IgE molecule:test compound complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . added if required, and the substrate is submitted to a detection device for analysis. Preferred test compounds to detect are ***Fc68*** R from any animal, antigens or anti-IgE antibodies.

SUMM . . . a complex between the feline IgE molecule and the test compound. Preferably, the feline IgE molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline IgE molecule is removed, a developing agent is. . . added if required, and the substrate is submitted to a detection device for analysis. Preferred test compounds to detect are ***Fc*** . ***epsilon*** . ***receptor*** from any animal, antigens or antiIgE antibodies.

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a calorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . .

SUMM . . . constant region is preferred. More preferred is a method as above, wherein the portion of the IgE molecule is the ***Fc*** . ***epsilon*** . ***receptor*** -binding region. Most preferred is a method as above wherein the portion of the IgE molecule is SEQ ID NO 2,. . .

SUMM . . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a feline IgE protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

DETD . . . feline IgE and do not bind to IgG or IgM. These five antibodies also do not react with canine or ***human*** IgE. In addition, three monoclonal antibodies reactive to feline IgE light chain were produced and are referred to as H-99,. . .

CLM What is claimed is:

2. An isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a feline IgE ***Fce*** receptor binding region and which comprises SEQ ID NO 4.

3. An isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a feline IgE ***Fce*** receptor binding region and which comprises SEQ ID NO 7.

4. An isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a feline IgE ***Fce*** receptor binding region and which comprises SEQ ID NO 10.

9. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fce*** receptor and comprises SEQ ID NO 11.

10. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fce*** receptor and comprises SEQ ID NO 8.

11. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fce*** receptor and comprises SEQ ID NO 5.

12. An isolated protein of claim 8, wherein said protein is capable of binding to ***Fce*** receptor and comprises SEQ ID NO 14.

17. A method to identify the ability of a test compound to interfere with IgE/ ***Fce*** receptor interaction, comprising: contacting the test compound with a protein of claim 8; and determining whether the test compound and. . .

L13 ANSWER 9 OF 24 USPATFULL on STN
AN 2003:3467 USPATFULL
TI Three-dimensional model of a complex between a ***Fc*** ***epsilon*** ***receptor*** alpha chain and a ***Fc*** region of an IgE antibody and uses thereof
IN Jardetzky, Theodore S., Chicago, IL, UNITED STATES
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PI US 2003003502 A1 20030102
AI US 2001-809715 A1 20010314 (9)
PRAI US 2000-189853P 20000315 (60)
DT Utility
FS APPLICATION
LREP HESKA CORPORATION, INTELLECTUAL PROPERTY DEPT., 1613 PROSPECT PARKWAY, FORT COLLINS, CO, 80525
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 14 Drawing Page(s)
LN.CNT 10630
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes three-dimensional models of complexes between antibody receptor proteins, such as ***Fc*** .epsilon.RI.alpha. proteins, and antibodies, such as ***Fc*** -C.epsilon.3/C.epsilon.4 regions of IgE antibodies, as well as methods to produce such models. The present invention also includes muteins having increased stability and/or antibody binding activity, as well as methods to produce such muteins, preferably using information derived from three-dimensional models of the present invention. Also included are nucleic acid sequences encoding muteins of the present invention and use of those sequences to produce such muteins. Also included is the use of the model to identify compounds that inhibit the binding of an antibody receptor protein to an antibody. The present invention also includes uses of such muteins and inhibitory compounds, for example, in methods to diagnose and protect animals from allergy and other abnormal immune responses.
TI Three-dimensional model of a complex between a ***Fc*** ***epsilon*** ***receptor*** alpha chain and a ***Fc*** region of an IgE antibody and uses thereof
AB The present invention includes three-dimensional models of complexes between antibody receptor proteins, such as ***Fc*** .epsilon.RI.alpha. proteins, and antibodies, such as ***Fc*** -C.epsilon.3/C.epsilon.4 regions of IgE antibodies, as well as methods to produce such models. The present invention also includes muteins having increased. . .
SUMM . . . invention relates to a crystal and a three-dimensional (3-D) model of a complex between a Fe epsilon receptor alpha chain (***Fc*** .epsilon.RI.alpha., or ***FceRIa***) protein and a constant region of an IgE antibody that includes the C.epsilon.3 and C.epsilon.4 domains (***Fc*** -C.epsilon.3/C.epsilon.4, or ***Fc*** -C.epsilon.3/C.epsilon.4, region). The present invention also relates to the use of that model to produce muteins and inhibitors useful in the. . .
SUMM [0004] Antibody ***Fc*** -receptors (***FcRs***) play an important role in the immune response by coupling the specificity of secreted antibodies to a variety of cells of the immune system. A number of cell types, including macrophages, mast cells, eosinophils, and basophils, express membrane-bound ***FcRs*** at their surfaces. The binding of antibodies to ***FcRs*** provides antigen-specificity to

these cells, which upon activation release further cell-specific mediators of the immune response, such as interleukins, initiators of inflammation, leukotrienes, prostaglandins, histamines, or cytotoxic proteins. The adoptive specificity of the ***FcRs*** allows a combinatorial approach to pathogen elimination, by coupling the diversity of antibody antigen-recognition sites to the variety of cell-types. . .

SUMM [0005] ***FcR*** -initiated mechanisms are important in normal immunity to infectious disease as well as in allergies, antibody-mediated tumor recognition, autoimmune diseases, and. . . other diseases in which immune responses are abnormal (i.e., not regulated). Recent experiments with transgenic mice have demonstrated that the ***FcRs*** control key steps in the immune response, including antibody-directed cellular cytotoxicity and inflammatory cascades associated with the formation of immune complexes; see, for example, Ravetch et al., 1998, Annu Rev Immunolo 16, 421-432. Receptors that bind IgG (***FcgRI*** , ***FcgRII*** , and ***FcgRIII*** , known collectively as ***FcgRs***) mediate a variety of inflammatory reactions, regulate B-cell activation, and also trigger hypersensitivity reactions. The high affinity ***Fc*** ***epsilon*** ***receptor*** (also known as the IgE receptor or ***FceRI***) is associated with the activation of mast cells and the triggering of allergic reactions and anaphylactic shock. Knockout mice for the ***FceRI*** alpha chain (***Fc*** .epsilon.RI.alpha.) are unable to mount IgE-mediated anaphylaxis (see for example, Dombrowicz et al., 1993, Cell 75, 969-976), although ***FcgRs*** are still able to activate mast cells (see, for example, Dombrowicz et al., 1997, J. Clin. Invest. 99, 915-925; Oettgen et al., 1994, Nature 370, 367-370). ***FceRI*** has also been shown to trigger anti-parasitic reactions from platelets and eosinophils as well as deliver antigen into the MHC. . . et al., 1997, Eur. J. Immunol. 27, 2212-2218; Maurer et al., 1998, J. Immunol. 161, 2731-2739. The beta subunit of ***FceRI*** has been associated with asthma in genetic studies; see, for example, Hill et al., 1996, Hum. Mol. Genet. 5, 959-962;. . . (.about.20%) may be affected by allergies, and this century has seen a substantial increase in asthma. Since IgE binding to ***FceRI*** is a requisite event in the reaction to different allergens, therapeutic strategies aimed at inhibiting ***FceRI*** could provide a useful treatment for these diseases. For example, monoclonal antibodies that target IgE and block receptor binding have. . .

SUMM [0006] ***FceRI*** is found as a tetrameric (abg.sub.2) or trimeric (ag.sub.2) membrane bound receptor on the surface of mast cells, basophils, eosinophils, langerhans cells and platelets. The alpha chain, also referred to as ***Fc*** .epsilon.RI.alpha., of ***FceRI*** binds IgE molecules with high affinity (K.sub.D of about 10.sup.-9 to 10.sup.-10 moles/liter (M)), and can be secreted as a. . . 266, 2639-2646, which describes the secretion of a soluble IgE-binding fragment of 172 amino acids. The extracellular domains of the ***human*** ***Fc*** .epsilon.RI.alpha. protein belong to the immunoglobulin (Ig) superfamily and contain seven N-linked glycosylation sites. Glycosylation of ***Fc*** .epsilon.RI.alpha. affects the secretion and stability of the receptor, but is not required for IgE-binding; see, for example, LaCroix et al.,. . . 1993, J. Biol. Chem. 268, 12736-12743; Scarselli et al., 1993, FEBS Lett 329, 223-226. The beta and gamma chains of ***FceRI*** are signal transduction modules.

SUMM [0007] Prior investigators have disclosed the nucleic acid sequence for ***human*** ***Fc*** .epsilon.RI.alpha.; see, for example, U.S. Pat. No. 4,962,035, by Leder, issued Oct. 9, 1990; U.S. Pat. No. 5,639,660, by Kinet et. . . 85, 1907-1911; and Pang et al., 1993, J. Immunol. 151, 6166-6174. Nucleic acid sequences have also been reported for the ***human*** ***FceRI*** beta and gamma chains; see, respectively, Kuster et al., 1992, J. Biol. Chem. 267, 12782-12787; Kuster et al., 1990, J. Biol. Chem. 265, 6448-6452. Nucleic acid sequences have also been reported for nucleic acid molecules encoding canine ***Fc*** .epsilon.RI.alpha., murine ***Fc*** .epsilon.RI.alpha., rat ***Fc*** .epsilon.RI.alpha., feline ***Fc*** .epsilon.RI.alpha. and equine ***Fc*** .epsilon.RI.alpha. proteins; see, respectively, GenBank.TM. accession number D16413; Swiss-Prot accession number P20489 (represents encoded protein sequence); GenBank accession number J03606; PCT. . . al., published

Aug. 5, 1999, referred to herein as WO 99/38974. In addition, methods to detect IgE antibodies using a ***Fc*** .epsilon.RI.alpha. protein have been reported in PCT Publication No. WO 98/23964, by Frank et al., published Jun. 4, 1998, referred to. . .

SUMM . . . have been several reports of the use of mutagenesis and swapping techniques to attempt to identify amino acids of either ***Fc*** .epsilon.RI.alpha. or IgE involved in the binding of (i.e., interaction between) those respective proteins, reports attempting to model ***Fc*** .epsilon.RI.alpha. proteins based on homology to other Ig-superfamily members, and reports that identify compounds that apparently inhibit such binding; see, for. . . No. WO 95/14779, by Gould et al., published Jun. 1, 1995. None of these references, however, describe isolated crystals of ***Fc*** .epsilon.RI.alpha. proteins or 3-D models derived from crystals.

SUMM [0009] Despite what is known about ***FcRs*** and their interaction with antibodies, there remains a need for ***FcRs*** and antibodies with improved characteristics, such as enhanced affinity for their ligands, altered substrate specificity, increased stability, and increased solubility. . .

SUMM [0010] The present invention includes isolated crystals of a complex between the extracellular domains of antibody receptor proteins (***FcRs***) and constant regions (***Fc*** regions) of antibodies, three-dimensional (3-D) models of such crystals and modifications of such models. The present invention also includes compounds that inhibit the ability of ***FcRs*** to bind to antibodies as well as ***FcR*** muteins and other modified ***FcRs*** as well as antibody muteins and other modified antibodies. Also included in the present invention are methods to produce and use such crystals, models, inhibitory compounds, muteins, and other modified proteins. As such, the present invention includes ***FcRs*** and antibodies with improved functions such as increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, and increased solubility, including but not limited to reduced aggregation. Such proteins, also. . .

SUMM [0011] The present invention includes a 3-D model of a complex between an extracellular domain of a ***human*** high affinity ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.RI.alpha.) protein and a ***human*** IgE ***Fc*** region comprising C.epsilon.3 and C.epsilon.4 domains, wherein the model substantially represents the atomic coordinates specified in Table 1. The present. . .

SUMM [0012] The present invention also includes an isolated crystal of a complex between an extracellular domain of a ***human*** high affinity ***Fc*** ***epsilon*** ***receptor*** alpha chain protein and a ***human*** IgE ***Fc*** region comprising C.epsilon.3 and C.epsilon.4 domains.

SUMM . . . The present invention includes a method to identify a compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein. The method includes the step of using a 3-D model of the present invention, and particularly one substantially represents. . .

SUMM [0014] The present invention also includes a mutein that binds to a ***Fc*** domain of an antibody or to a ***Fc*** binding domain of a ***FcR*** . Such a mutein has an improved function compared to a protein that includes SEQ ID NO:2 or SEQ ID NO:6, respectively. Examples of such an improved function include increased stability, increased affinity for an ***Fc*** domain of an antibody, altered substrate specificity, decreased aggregation, and increased solubility. Such a mutein is produced by a method. . . having such an improved function. The present invention also includes a mutein having an improved function compared to an unmodified ***Fc*** .epsilon.RI.alpha. protein or IgE ***Fc*** region.

SUMM [0015] Also included are muteins that are chemically modified ***Fc*** .epsilon.RI.alpha. proteins or antibodies. Also included are nucleic acid molecules that encode muteins of the present invention, recombinant molecules and recombinant. . .

SUMM [0016] The present invention also includes a method to improve a function of a ***Fc*** .epsilon.RI.alpha. protein or IgE ***Fc*** region which includes the steps of: (a) analyzing a 3-D model substantially representing the atomic coordinates specified in Table 1. . .

DRWD [0017] FIG. 1 shows an electron density map and ribbon diagrams depicting the overall structure of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex. FIG. 1 a shows a stereo diagram from a .sigma..sub.a-weighted 2F.sub.o-F.sub.c simulated annealing omit electron density map at 3.5 angstroms. The complex is contoured at 1.25.sigma.. ***Fc*** .epsilon.RI.alpha. residues 129-136 of ***Fc*** .epsilon.RI.alpha. and IgE- ***Fc*** loop residues 334-336 and 362-364 are shown. FIG. 1b is a side view of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex depicting the two ***Fc*** chains (yellow and red ribbon, upper left of figure) and the ***Fc*** .epsilon.RI.alpha. chain (blue ribbon, lower right of figure). Binding sites 1 and 2 are indicated. The cell membrane would lie below the receptor. FIG. 1c is a top view of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex shown in FIG. 1b.

DRWD [0018] FIG. 2 shows a surface representation of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex. FIG. 2a is a side view of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex highlighting how the convex surface of the receptor interacts asymmetrically with the two IgE- ***Fc*** C.epsilon.3 domains. The two ***Fc*** chains are in yellow and red while the ***Fc*** .epsilon.RI.alpha. chain is in blue. Carbohydrate surfaces are white, detergent surface is black. FIG. 2b is a top view of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex surface representation shown in FIG. 2a. FIG. 2c is a superposition of the two IgE- ***Fc*** C.epsilon.3 domains. The twofold symmetry of the IgE- ***Fc*** domains is broken in the C.epsilon.2-C.epsilon.3 linker region (residues 328-336) by interactions with the receptor. Superposition of the C.epsilon.3 domains. . . domain, because of a 3.degree. difference in C.epsilon.3 and C.epsilon.4 pseudo-dyad axes. FIG. 2d is a surface representation of both IgE- ***Fc*** and ***Fc*** .epsilon.RI.alpha. in which the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex has been separated to expose the surfaces involved in binding. The IgE (upper left) is oriented to give an end-on view of the C.epsilon.3 domains. Binding residues that bind ***Fc*** .epsilon.RI.alpha. are shown in yellow (Site 1) and red (Site 2). A top and side view of the ***Fc*** .epsilon.RI.alpha. is shown on the right-hand side of FIG. 2d. Residue Y131 of site 1 and the binding pocket for P426 of the IgE- ***Fc*** are labeled. Carbohydrate is shown in grey.

DRWD [0019] FIG. 3 details the interactions in the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex at Site 1 and Site 2. FIG. 3a is a plot showing the buried surface area of residues in the IgE- ***Fc*** .epsilon.3 domains. The top half of the graph shows residues buried in the Site 1 interaction (yellow bars), while the bottom. . . N394 is due to attached carbohydrate. FIG. 3b is a stick model diagram of residue interactions at Site 1. The IgE- ***Fc*** and ***FcIa*** chains are tan and blue, respectively. Binding loops are labeled at their termini, side chains of residues buried in the. . . shown and Y131 is labeled. FIG. 3c is a stick model diagram of the residue interactions at Site 2. The IgE- ***Fc*** and ***Fc*** .epsilon.RI.alpha. chains are red and blue, respectively. Side chains of residues buried in the complex are shown. FIG. 3d is a space filling model showing binding of CHAPS detergent molecule in the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex. Atoms less than 4A apart have dotted lines between them and the residues are labeled. No density appears for. . .

DRWD [0020] FIG. 4 illustrates the conservation of amino-acid residues and contacts at the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. interfaces in IgG receptors and antibodies. Contacting residues are defines as interatomic distances <4 .ANG.. FIG. 4a shows the Site 1 interacting residues and their conservation in related ***human*** receptors and antibodies. Absolutely conserved residues are highlighted in bold and partially conserved residues are lightly highlighted (yellow for IgE, blue for ***Fc*** .epsilon.RI.alpha.). Dark lines are drawn for residues making the largest number of contacts across the interface, lighter lines for intermediate number. . . contacts, and dashed lines for the fewest contacts. FIG. 4b shows the Site 2 interacting residues and their conservation in ***human*** related ***Fc*** receptors and antibodies. Receptor residues are highlighted in blue, antibody residues in red. Three residues in IgG2 (PVA) that disrupt binding to ***Fc*** .gamma.RI are boxed in black. FIG. 4c is a closeup of the Site 2 trp/proline interaction (***FcR*** surface with IgE-ribbon

interaction). Also shown are residues implicated in the IgG specificity between different receptor subtypes (corresponding to residues 332-334 in IgE) that interact with the FG loop. FIG. 4d is shows how ***FcRY131*** in Site 1 interacts with a shallow pocket on the C.epsilon.3 domain that could be a source of specificity for IgG interactions (Y changes to H or R in ***Fc*** .gamma.RII and ***Fc*** .gamma.RIII).

DRWD . . . scheme for the binding of IgE to its receptor. The interaction of each C.epsilon.3 domain with distinct surfaces of the ***Fc*** .epsilon.RI.alpha. structure suggests a kinetic scheme in which transient release of one of the C.epsilon.3 domains may occur within the complex.. . .

DRWD [0022] FIG. 6 is a ribbon-model showing the superposition of the ***Fc*** portion of an intact IgG antibody (1IGY)27 and IgG ***Fc*** receptor ***Fc*** .gamma.RII22 onto the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex. The IgE complex is shown in beige and the IgG homologues in blue. Only a minor adjustment of the. . .

DRWD [0023] FIG. 7 shows a hypothetical model for an intact IgE: ***Fc*** receptor complex. The ***Fc*** chains are in red and yellow, the ***Fc*** .epsilon.RI.alpha. chain is in blue. Antibody Fab regions are shown in beige.

DETD [0024] The present invention includes isolated crystals of complexes between the extracellular domains of ***FcRs*** and ***Fc*** regions of antibodies, 3-D models of such crystals and modifications of such models. The present invention also includes compounds that inhibit the ability of ***FcRs*** to bind to antibodies as well as muteins and other modified ***FcRs*** and antibodies. Also included in the present invention are methods to produce and use such crystals, models, inhibitory compounds, muteins,. . .

DETD [0025] The present invention includes an isolated crystal of a complex between an extracellular domain of a high affinity ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.RI.alpha.) and a ***Fc*** region comprising the C.epsilon.3 and C.epsilon.4 domains of an IgE antibody (***Fc*** -C.epsilon.3/C.epsilon.4), a 3-D model of such a crystal and a modification of such a model. As used herein, the term "a". . .

DETD [0026] As used herein, an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein is the portion of the ***FceRI*** alpha chain that is exposed to the environment outside the cell and that binds to the ***Fc*** domain of an IgE antibody. Such an extracellular domain can be (a) a complete extracellular domain which is a domain that extends from the first amino acid of a mature ***FceRI*** alpha chain through the last amino acid prior to the start of the transmembrane region or a domain that is. . . a domain includes a D1 and D2 domain, displays a similar affinity for the IgE antibody to which such an ***Fc*** .epsilon.RI.alpha. protein naturally binds, and produces crystals having sufficient quality to enable structure determination, or (b) a fragment of any of the extracellular domains of (a), wherein the fragment retains its ability to bind to the ***Fc*** domain of an antibody. As used herein, the terms binding to an antibody and binding to the ***Fc*** domain (i.e., constant region) of an antibody can be used interchangeably since it is recognized that a ***FcR*** binds to the ***Fc*** domain of an antibody. A ***FcR*** (i.e., a protein that can bind to an antibody), such as a ***Fc*** .epsilon.RI.alpha. protein, can be a full-length ***FcR*** (e.g., a full-length ***FceRI*** alpha chain), or any fragment thereof, wherein the fragment binds to an antibody. Similarly an antibody, or an ***Fc*** region thereof, can be a full-length antibody, or full-length ***Fc*** region thereof, or any fragment thereof that binds to a ***FcR*** . In one embodiment an ***Fc*** region comprises C.epsilon.3 and C.epsilon.4 domains. Preferably a ***FcR*** binds to an antibody with an affinity (K.sub.A) of at least about 10.sup.8 liters/mole (M.sup.-1), more preferably of at least. .

DETD . . . For example, this is the first report of an isolated crystal of a complex between an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4 region of an IgE antibody, and in particular of an isolated crystal of sufficient quality that a crystal structure, i.e.,. . . difficult and non-obvious and has been attempted by others without success. The inventors tried many approaches before discovering a preferred

Fc .epsilon.RI.alpha. protein and a preferred ***Fc*** -C.epsilon.3/C.epsilon.4 region from which to make a useful crystal. Part of the reason for the difficulty is that the ***Fc*** .epsilon.RI.alpha. protein is highly glycosylated. Although crystals could be produced using a ***Fc*** .epsilon.RI.alpha. protein that consists of amino acids 1 through 176 of the mature ***human*** ***Fc*** .epsilon.RI.alpha. protein, a protein that is denoted herein as PhFc.epsilon.RI.alpha..sub.1-176, or the hFc.epsilon.RI.alpha..sub.1-176 protein, and has an amino acid sequence denoted herein as SEQ ID NO:2, much better crystals could be generated using a ***Fc*** .epsilon.RI.alpha. protein that consists of amino acids 1 through 176 of the mature ***human*** ***Fc*** .epsilon.RI.alpha. protein that had been mutated to replace four N-linked glycosylation sites with other amino acids at positions 74, 135, 142. . . herein as nhFc.epsilon.RI.alpha..sub.1-528mut, the nucleic acid sequence of which is denoted herein as SEQ ID NO:3. Identification of an appropriate ***Fc*** -C.epsilon.3/C.epsilon.4 region to crystallize was also difficult. The first such region to be used successfully is referred to herein as PhFc-C.epsilon.3/C.epsilon.4.sub.1-222. . . acids alanine, aspartic acid, proline and cysteine at the amino terminus followed by amino acids 330 through 547 of the ***human*** IgE ***Fc*** constant region, using the numbering system of Dorrington et al, 1978, Immunol Rev 41, 3-25. PhFc-C.epsilon.3/C.epsilon.4.sub.1-222 is represented herein by. . .

DETD . . . PhFc.epsilon.RI.alpha..sub.1-176mut and PhFc-C.epsilon.3/C.epsilon.4.sub.1-222, a number of other proteins were tried without success, as described in the Examples. , including a ***Fc*** .epsilon.RI.alpha. protein spanning from amino acid 1 through 171 of SEQ ID NO:2 produced in Pichia pastoris, and ***Fc*** .epsilon.RI.alpha. proteins spanning from amino acid 1 through 172 of SEQ ID NO:2 produced in Chinese hamster ovary cells, Trichoplusia ni. . .

DETD . . . proteins containing immunoglobulin domains, herein also referred to as Ig domains, but also in view of the crystal structures of ***Fc*** .epsilon.RI.alpha. alone, which is disclosed in U.S. patent application Ser. No. 09/434,193, filed Nov. 4, 1999, by Jardetzky et al., and in PCT Publication No. WO 00/26246, published May 11, 2000, by Jardetzky et al., and of ***Fc*** -C.epsilon.3/C.epsilon.4 alone, which is disclosed in U.S. patent application Ser. No. 60/189,403, filed Mar. 15, 2000, by Jardetzky et al. WO. . . ibid., 09/434,193, ibid., and 60/189,403, ibid. are incorporated by reference herein in their entireties. Not only is the structure of ***Fc*** .epsilon.RI.alpha. in the complex fairly similar to the unique structure of ***Fc*** .epsilon.RI.alpha. alone, but, even more surprisingly, the structure of ***Fc*** -C.epsilon.3/C.epsilon.4 in the complex is very different from that of ***Fc*** -C.epsilon.3/C.epsilon.4 alone. For example, as disclosed in 60/189,403, ibid., the ***Fc*** region of IgE alone exists in a closed conformation whereas receptor-bound IgE ***Fc*** exists in an open conformation. The model also predicts that a ***Fc*** .epsilon.RI.alpha. protein and an IgE ***Fc*** region bind at a stoichiometry of 1:1 which is surprising since each ***Fc*** region has two C.epsilon.3 domains. Comparison of these structural similarities and differences are described in greater detail in the Examples.. . . mutagenesis and region swapping studies that have been reported. Such a model permits differentiation, even more so than models of ***Fc*** .epsilon.RI.alpha. alone as disclosed in Ser. No. 09/434,193, ibid., WO 00/26246, ibid., and Garman et al., 1999, Cell 95, 951-961, between amino acids directly or indirectly influencing binding of IgE to ***Fc*** .epsilon.RI.alpha. and demonstrates where amino acids and amino acid segments identified in mutagenesis and swapping studies are positioned on the protein. By using a model of the present invention one can identify the interactions of ***Fc*** .epsilon.RI.alpha. and IgE, thereby identifying amino acids to target for mutein production or regions to target for the development of compounds. . . binding of IgE to its receptor. Such a model can be used alone or in conjunction with a model of ***Fc*** .epsilon.RI.alpha. alone (Ser. No. 09/434,193, ibid. or WO 00/26246, ibid.) or ***Fc*** -C.epsilon.3/C.epsilon.4 alone (60/189,403, ibid.).

DETD [0030] One embodiment of the present invention is an isolated crystal of a complex between an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4

region of an IgE antibody. As used herein, an isolated crystal is a crystal of a protein that has been. . . precipitants are known to those skilled in the art. In a preferred embodiment, a crystal of a complex between an ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4 region is produced in a solution by adding a precipitant such as polyethylene glycol (PEG) or PEG monomethylether. In one. . . produced in the presence of 3-[3-(cholamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS), or a similar detergent. It is also to be noted that a ***Fc*** .epsilon.RI.alpha. protein and ***Fc*** -C.epsilon.3/CC.epsilon.4 region used to produce a crystal can be produced by a variety of methods, including purification of a native protein,. . .

DETD . . . present invention are not derivatized. In one embodiment, an isolated crystal of the present invention is a co-crystal of a ***Fc*** .epsilon.RI.alpha. protein bound to a ***Fc*** domain of an IgE antibody in the presence of a compound that inhibits the binding of a ***Fc*** .epsilon.RI.alpha. protein to a ***Fc*** domain of an IgE antibody. Additional crystals of the present invention include crystals produced from proteins that are muteins of. . .

DETD . . . isolated crystal of the present invention can be the crystal of a complex between any suitable extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** region that binds to ***Fc*** .epsilon.RI.alpha., such as a Fe comprising C.epsilon.3 domains or a Fe comprising C.epsilon.3 and C.epsilon.4 domains. Suitable ***Fc*** .epsilon.RI.alpha. proteins include mammalian ***Fc*** .epsilon.RI.alpha. proteins, with ***human*** , canine, feline, equine, rat and murine ***Fc*** .epsilon.RI.alpha. proteins being preferred, and ***human*** ***Fc*** .epsilon.RI.alpha. proteins being even more preferred. Suitable ***Fc*** -C.epsilon.3/C.epsilon.4 regions include mammalian ***Fc*** -C.epsilon.3/C.epsilon.4 regions, proteins, with ***human*** , canine, feline, equine, rat and murine ***Fc*** -C.epsilon.3/C.epsilon.4 regions being preferred, and ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4 regions being even more preferred. A preferred crystal of the present invention diffracts X-rays to a resolution of about 4.5. . . NO:2, amino acid sequence SEQ ID NO:4, or a sequence essentially equivalent that represents an extracellular domain of another mammalian ***Fc*** .epsilon.RI.alpha. protein in complex with a ***Fc*** -C.epsilon.3/C.epsilon.4 region having amino acid sequence SEQ ID NO:6, or a sequence essentially equivalent that represents another mammalian Fe-C.epsilon.3/C.epsilon.4 region. Preferred. . .

DETD [0033] The present invention includes a 3-D model of a complex between an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4 region that substantially represents the atomic coordinates specified in Table 1. The present invention also includes 3-D models that comprise. . . the model substantially represented by the atomic coordinates specified in Table 1. Each such modification represents a complex between a ***Fc*** receptor protein that binds to a ***Fc*** domain of an antibody and an antibody ***Fc*** region that binds to a ***Fc*** receptor protein. A 3-D model of a complex between an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4 region is a representation, or image, that predicts the actual structure of the corresponding complex. As such, a 3-D model. . . relationship between the complex's structure and function at the atomic level and to design muteins (i.e., genetically and/or chemically altered ***FcRs*** or antibodies) having an improved function, such as, but not limited to: increased (i.e., enhanced) stability; increased antibody or ***FcR*** , respectively, binding activity, for example, by, increasing the affinity for an antibody or ***FcR*** , respectively, by, for example, increasing the association rate and/or decreasing the dissociation rate between a ***FcR*** and an antibody or by altering substrate specificity (e.g., enhancing the ability of a ***FcR*** of a certain species and class to bind to antibody from another species and/or another antibody class); and/or increased solubility. . . model can be subjected to further refinements to more closely correspond to the actual structure of a complex between a ***FcR*** and antibody. Such a refined model, which is an example of a modification of the present invention, is a better. . . 3-D model of the present invention refers to an improved model of a complex between an extracellular domain of a ***Fc***

.epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4 region that can be obtained in a variety of ways known to those skilled in the art. Refinements can include. . .

DETD [0034] One embodiment of the present invention is a 3-D model of a complex between an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4 region that substantially represents the atomic coordinates specified (i.e., listed) in Table 1.

TABLE 1

Atomic coordinates of coml4i_deposit.pdb

ATOM ATOM

#. . .

DETD . . . to herein as a model modification, is a model that represents a complex between a protein that binds to a ***Fc*** domain of an antibody and an antibody ***Fc*** regon that binds to a ***Fc*** receptor protein. A model modification includes, but is not limited to: a refinement of the model that substantially represents the atomic coordinates specified in Table 1; a model representing a complex between any ***Fc*** -binding fragment of a ***Fc*** receptor protein and any ***FcR*** -binding fragment of an antibody having the atomic coordinates specified in Table 1; a model based on other ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 crystals, such as a model based on one or more of the crystals disclosed in the Examples; a model produced using homology modeling techniques to, for example, incorporate all or any part of the amino acid sequence of another ***FcR*** or antibody into a 3-D model substantially representing the atomic coordinates specified in Table 1 or incorporate all or any part of the amino acid sequence of a ***Fc*** .epsilon.RI.alpha. protein or ***Fc*** -C.epsilon.3/C.epsilon.4 into a 3-D model of a complex between another ***FcR*** and antibody; and a modification representing a complex between an ***FcR*** and antibody, at least one of which has an altered function, which preferably can be used to design a mutein. . .

DETD . . . for example by extracting coordinates from a picture or placing a similar immunoglobulin domain into the 3-D model of a ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein having SEQ ID NO:2 and deriving a model of the similar domain. Physical 3-D models are tangible and include,. . .

DETD . . . mold, or alpha-space, that can be used to predict the shape of a compound that inhibits the binding of a ***FcR*** and antibody.

DETD . . . on the external surface of the proteins in the complex and, as such, may be involved in binding of a ***FcR*** to an antibody and as such be useful in designing proteins with an enhanced binding activity or in identifying compounds that inhibit such binding. In addition, solvent accessible residues can represent targets for modification to produce a ***FcR*** or antibody with improved function. Such analysis also identifies residues in the interior, or core, of the proteins in the. . .

DETD . . . crystals and predict the location of the IgE binding domain, including those amino acids that actually form contacts with a ***Fc*** domain of an IgE antibody, such as those in the binding face of the ***Fc*** .epsilon.RI.alpha. protein. A model can also identify the amino acids in the interface between domain 1 and domain 2 (i.e., the D1D2 interface), as well as those in the cleft formed between the two domains of the ***Fc*** .epsilon.RI.alpha. protein. Particularly important regions of the complex indicated by the model represented in Table 1 include, but are not limited to, ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1, ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 2, the hinge between domain C.epsilon.3 and domain C.epsilon.4 of the ***Fc*** -C.epsilon.3/C.epsilon.4 region, and a ***FcERIa*** : ***Fc*** -C.epsilon.3/C.epsilon.4 region that interacts with 3-[3-(cholamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS). Interaction sites 1 and 2 are the sites at which amino acids from ***Fc*** .epsilon.RI.alpha. and ***Fc*** -C.epsilon.3/C.epsilon.4 interact with each other. These sites are described in more detail in the Examples and represent sites to target for. . .

DETD . . . embodiment of the present invention is a model that represents a complex that includes a protein that binds to a ***Fc*** domain of

an IgE antibody with an affinity that is at least equivalent to the affinity of the extracellular domain of ***human*** ***Fc*** .epsilon.RI.alpha. for any one of the following IgE antibodies: a ***human*** IgE antibody, a canine IgE antibody, a feline IgE antibody, an equine IgE antibody, a rat IgE antibody, and a murine IgE antibody. Such a model can represent an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, a canine ***Fc*** .epsilon.RI.alpha. protein, a feline ***Fc*** .epsilon.RI.alpha. protein, an equine ***Fc*** .epsilon.RI.alpha. protein, a murine ***Fc*** .epsilon.RI.alpha. protein, and a rat ***Fc*** .epsilon.RI.alpha. protein. Such a model can also represent a protein with altered substrate specificity, preferably designed based on a model of the present invention. WO 98/23964, ibid., reports the ability of an isolated ***human*** ***Fc*** .alpha.RI.alpha. protein to bind to canine, feline and equine IgE antibodies. Models of the present invention can be used to design a ***FcR*** with increased affinity for an antibody of a species other than self, such as, but not limited to, a ***human*** ***Fc*** .epsilon.RI.alpha. with increased affinity for a canine, feline or equine IgE antibody.

DETD [0043] A model of the present invention can also represent a complex that includes a ***Fc*** domain of an antibody that binds to a ***Fc*** .epsilon.RI.alpha. protein with an affinity that is at least equivalent to the affinity of a ***human*** IgE antibody ***Fc*** -C.epsilon.3/C.epsilon.4 region for the extracellular domain of any of the following ***Fc*** .epsilon.RI.alpha. proteins: a ***human*** ***Fc*** .epsilon.RI.alpha. protein, a canine ***Fc*** .epsilon.RI.alpha. protein, a feline ***Fc*** .epsilon.RI.alpha. protein, an equine ***Fc*** .alpha.RI.alpha. protein, a murine ***Fc*** .epsilon.RI.alpha. protein and a rat ***Fc*** .epsilon.RI.alpha. protein. Such a model can represent a ***FceRI*** -binding domain of a ***human*** , canine, feline, equine, murine or rat Fe region. Such a model can also represent a Fe region with altered substrate. . .

DETD [0044] The present invention includes a model that represents a complex between a ***FcR*** and a Fe domain that binds to an antibody or receptor of its respective class (i.e., IgE, IgG, IgM, IgA or IgD antibody class or corresponding Fe receptor). Also included is a model that represents a complex between a ***FcR*** and antibody designed to bind to an antibody or receptor, respectively, of a class other than the class to which. . . invention can be produced, for example, by incorporating all or any part of the amino acid sequence of the other ***FcR*** or antibody into a 3-D model substantially representing the coordinates in Table 1. Such an embodiment includes any model that. . . domains that are placed in an orientation (packing interfaces and bend angles) that is based on the structure of the ***Fc*** .epsilon.RI or a model that is based on the 1:1 stoichiometry predicted by the coordinates in Table 1. A preferred model of the present invention represents a complex including a ***FcR*** that binds to an IgE antibody or to an IgG antibody. In one embodiment, a model of the present invention is a 3-D model of a complex between an extracellular antibody binding domain of a ***FcR*** other than ***human*** ***Fc*** .epsilon.RI.alpha., such as of a ***FcR*** that binds to an IgG antibody and an antibody. Such proteins and models thereof can be designed by homology modeling by, for example, altering the substrate specificity of a ***Fc*** .epsilon.RI.alpha. protein such that the altered protein binds an IgG antibody.

DETD . . . 3-D model substantially represented by the atomic coordinates specified in Table 1. In this embodiment, such a model represents a ***FcR*** binding to an antibody. The backbone atoms are those atoms that form the backbone, or 3-D folding pattern, of the. . . amino acids, i.e., nitrogen, carbon, the alpha carbon and oxygen. Also preferred is a model modification that includes (a) a ***FcR*** protein having an amino acid sequence that shares at least about 30%, preferably at least about 40%, more preferably at. . . more preferably at least about 60% and even more preferably at least about 80% amino acid sequence homology, with a ***human*** ***Fc*** .epsilon.RI.alpha. protein, as determined using the program ALIGN with default parameters, optimal global alignment of two sequences with no short-cuts and (b) a ***Fc*** region having an amino acid sequence that shares at least about 30%, preferably at least about 40%, more preferably at. . . more preferably at least about 60% and even more

preferably at least about 80% amino acid sequence homology, with a ***Fc*** -C.epsilon.3/C.epsilon.4 region of a ***human*** IgE antibody, as determined using the program ALIGN with default parameters, optimal global alignment of two sequences with no short-cuts. It is to be noted that, using the same program and parameters, the extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein (i.e., soluble ***human*** ***Fc*** .epsilon.RI.alpha. protein) shares about 48% identity with feline and rat soluble ***Fc*** .epsilon.RI.alpha. proteins, about 49% with a murine soluble ***Fc*** .epsilon.RI.alpha. protein, about 50% identity with a canine soluble ***Fc*** .epsilon.RI.alpha. protein, and about 60% identity with an equine soluble ***Fc*** .epsilon.RI.alpha. protein. A preferred model of the present invention represents an IgE binding domain, i.e., a region that binds to an IgE antibody, complexed to a ***Fc*** .epsilon.RI.alpha.-binding domain, i.e., a region that binds to a ***Fc*** .epsilon.RI.alpha. protein.

DETD [0046] One embodiment of the present invention is a 3-D model of a complex between a ***human*** ***Fc*** .epsilon.RI.alpha. protein and a ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4 region produced by a method that includes the steps of: (a) crystallizing a complex between an extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein, such as, but not limited to a protein having amino acid sequence SEQ ID NO:2 or SEQ ID NO:4 and a ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4 region, such as, but not limited to a protein having amino acid sequence SEQ ID NO:6; (b) collecting X-ray diffraction. . . formation can be produced using a variety of techniques well known to those skilled in the art. As disclosed herein, ***human*** ***Fc*** .epsilon.RI.alpha. proteins and ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4 region sto be crystallized are preferably produced in recombinant insect cells transformed with a gene encoding the respective proteins, such as a baculovirus genetically engineered to produce the respective protein. The purity of the ***Fc*** .epsilon.RI.alpha. protein or ***Fc*** -C.epsilon.3/C.epsilon.4 region must be sufficient to permit the production of crystals that can be analyzed by X-ray crystallography to a resolution. . .

DETD [0047] As disclosed herein, a preferred method to crystallize a complex between a ***Fc*** .epsilon.RI.alpha. protein and a ***Fc*** -C.epsilon.3/C.epsilon.4 region is by vapor distillation. Particularly preferred methods are disclosed in the Examples. It should be appreciated that the present. . .

DETD . . . London, 1976. However, as discussed herein, elucidation of the crystal structure of a complex between the extracellular domain of the ***human*** ***Fc*** .epsilon.RI.alpha. and a ***Fc*** -C.epsilon.3/C.epsilon.4 region of a ***human*** IgE was difficult. In one embodiment, crystal structure determination includes obtaining high-resolution data using synchrotron radiation. Such data can be. . .

DETD [0049] One embodiment of the present invention is a method to produce a 3-D model of a ***Fc*** .epsilon.RI.alpha. protein that includes positioning amino acid representations (i.e., representing amino acids) of the protein at substantially the coordinates listed in. . .

DETD . . . frozen crystals; introduction of solvent molecules to the structure; clarification of secondary structure; and analyses of crystallized complexes between a ***FcR*** and an antibody or inhibitory compound or of crystallized ***FcRs*** or antibodies alone. An additional model refinement method includes analyzing a 3-D model to predict amino acid residues that if. . .

DETD [0051] Another embodiment of the present invention is a modified 3-D model that represents a complex between a ***FcR*** other than a ***human*** ***Fc*** .epsilon.RI.alpha. protein represented by the 3-D model the coordinates of which are listed in Table 1 and an antibody other than ***human*** IgE as represented by the coordinates in Table 1. Preferably the amino acid sequence of the protein(s) to be modeled. . . preferably by incorporating (e.g., grafting, overlaying or replacing) all or any portion of the amino acid sequence of the other ***FcR*** or antibody into the 3-D model representing the coordinates of Table 1 to produce the modified model. General techniques for. . . Genetics 8, 30-43; and Lattman, 1985, Methods Enzymol 115, 55-77. However, such technology has not been applied to complexes between ***FcRs*** and antibodies since, until the present invention, no 3-D

model of any ***FcR*** :antibody complex was available. Thus, the present invention now allows the solving of the structures of a number of other natural and mutated forms of ***FcRs*** , antibodies or complexes thereof.

DETD [0052] In one embodiment, a model of a ***FcR*** : ***Fc*** complex, such as, but not limited to a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 complex, is produced by extracting the 3-D coordinates from a published figure or building a 3-D model with atoms from other domains wherein the domain 1 and 2 of the ***FcR*** and ***FcR*** -binding domains of the antibody are oriented as predicted for a complex between the ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein and ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4.sub.222 protein. For example, a model of the present invention can be produced by orienting two known Ig domains into a bent confirmation similar to that of the two domains of the ***human*** ***Fc*** .epsilon.RI.alpha. protein. Such a model is referred to as a model in which domain 1 and domain 2 are oriented in. . .

DETD [0053] Suitable ***FcRs*** or antibodies for which a 3-D model can be determined using homology modeling include any mammalian ***FcR*** or antibody, such as a protein that binds to IgE, IgG, IgM, IgA or IgD antibodies or an antibody that binds to the corresponding ***FcR*** . Preferred is a ***FcR*** protein that binds to an IgE antibody or an IgG antibody. Preferred ***FcRs*** that bind to IgE include ***human*** , canine, feline, equine, murine and rat ***Fc*** .epsilon.RI.alpha. proteins. Preferred antibodies that bind to ***FcRs*** include ***human*** , canine, feline, equine, murine and rat antibodies. The present invention also includes the use of other Ig domains to produce. . .

DETD [0054] One embodiment of the present invention is a 3-D model of a ***FcR*** :antibody complex in which one or both proteins have an improved function compared to an unmodified protein as well as a. . . or the entire protein to, for example, introduce non-natural amino acids or other chemical compounds into the structure of a ***FcR*** or antibody. For example, based on a structure of the present invention, one can design synthetic peptides or larger proteins that could be linked to produce an intact protein with IgE or ***FcR*** binding activity, the structure allowing one to design the start and stop points for these peptides, e.g., at surface accessible. . .

DETD . . . invention includes use of a 3-D model of the present invention to identify a compound that inhibits binding between a ***FcR*** and an antibody. The advantages of using a 3-D model to identify inhibitory compounds are multi-fold in that the model depicts the site at which a ***Fc*** region of an antibody binds to its ***FcR*** , i.e., the antibody-binding domain, also referred to as the antibody binding site, and the ***FcR*** -binding domain, also referred to as the ***FcR*** binding site. The antibody binding site and the ***FcR*** binding site together form an ***FcR*** :antibody interaction site. As such, a large number of potential inhibitory compounds can be initially analyzed without having to perform in. . . to identify inhibitory compounds include, but are not limited to, designing inhibitory compounds based on the 3-D model of a ***FcR*** , probing such a 3-D model with compounds that are potential inhibitors in order to identify those compounds that are actually inhibitory of the binding of an antibody to its ***FcR*** , screening a compound data base using such a 3-D model to identify compounds that inhibit such binding, and combinations thereof.. . .

DETD [0056] An inhibitory compound can be any natural or synthetic compound that inhibits the binding of an antibody to a ***FcR*** . Examples include, but are not limited to, inorganic compounds, oligonucleotides, proteins, peptides, antibodies, antibody fragments, mimetics of peptides or antibodies. . . interact at the binding site or allosterically. An inhibitory compound should be capable of physically and structurally associating with a ***FcR*** and/or an antibody such that the compound can inhibit binding between the two entitites. As such, an inhibitory compound is. . . be identified in one or multiple steps. For example, a compound initially identified that inhibits binding between an antibody and ***FcR*** to some extent can be used as a lead to design, probe or screen for a compound with improved characteristics,. . .

DETD . . . the present invention is a method to identify a compound that

inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein. Such a method includes the step of using a 3-D model substantially representing the atomic coordinates specified in Table. . . binding domain or the receptor binding domain of the IgE antibody as well as compounds that interact indirectly with an ***Fc*** .epsilon.RI.alpha. protein, such as compounds that interact with the IgE binding domain, the ***Fc*** .epsilon.RI.alpha. binding domain, ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1, ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 2, the hinge between domain C.epsilon.3 and domain C.epsilon.4 of the ***Fc*** -C.epsilon.3/C.epsilon.4 region, or a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 region that interacts with CHAPS. In a preferred embodiment, an inhibitory compound interacts with at least one of the following regions of a model representing a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 complex: a C strand of domain 2 of ***Fc*** .epsilon.RI.alpha., a C'E loop of domain 2 of ***Fc*** .epsilon.RI.alpha., a tryptophan-containing hydrophobic ridgeof ***Fc*** .epsilon.RI.alpha., a linker between domain 1 and domain 2 of ***Fc*** .epsilon.RI.alpha., a BC loop of domain 2 of ***Fc*** .epsilon.RI.alpha., a FG loop of domain 2 of ***Fc*** .epsilon.RI.alpha., a C.epsilon.2/C.epsilon.3 linker region of ***Fc*** -C.epsilon.3/C.epsilon.4, a BC loop of ***Fc*** -C.epsilon.3/C.epsilon.4, a DE loop of ***Fc*** -C.epsilon.3/C.epsilon.4, and a FG loop of ***Fc*** -C.epsilon.3/C.epsilon.4. Inhibitory compounds of the present invention preferably interact with at least one of the following amino acids: (a) a residue. . . residues listed in (a) or (b). Particularly preferred amino acids with which to interact are: (a) a residue within the ***Fc*** .epsilon.RI.alpha. pocket for the proline at position 101 of SEQ ID NO:6, such residues including, but not limited to positions 85,. . . view of a model of the present invention. These regions and residues are a refinement of those identified using a ***Fc*** .epsilon.RI.alpha. model as described in 09/434,193, ibid. or WO 00/26246, ibid. In one embodiment, an inhibitory compound of the present invention. . .

DETD [0058] One embodiment of a method to identify a compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein includes the steps of: (a) generating a model substantially representing the atomic coordinates listed in Table 1 or of. . . a compound to be tested; and (c) testing to determine if the compound interacts with said IgE binding domain or ***FcR*** binding domain, wherein such an interaction indicates that the compound is capable of inhibiting the binding of an IgE antibody to a ***Fc*** .epsilon.RI.alpha. protein. In a preferred embodiment, step (a) includes the step of identifying one or more amino acid(s) in the IgE binding domain of ***FcR*** binding domain of the model that interact directly with the corresponding domain. Preferably a compound to be tested will interact. . .

DETD . . . of the present invention to expand the use of models of the present invention to produce models of any suitable ***FcRs*** (i.e., model modifications) and to identify compounds that inhibit the binding of antibodies to such ***FcRs*** .

DETD . . . 3-[3-(cholamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS) or a compound having a similar ring structure. The interaction of CHAPS with amino acids in the ***Fc*** .epsilon.RI.alpha. protein and ***Fc*** -C3/C4 region is described in further detail in the Examples.

DETD . . . invention also includes use of a 3-D model of the present invention to rationally design and construct modified forms of ***FcRs*** or antibodies that have one or more improved functions, such as, but not limited to, increased activity, increased stability and increased solubility compared to an unmodified ***FcR*** or antibody. Muteins of the present invention include full-length proteins as well as fragments (i.e., truncated versions) of such proteins.

DETD [0063] One embodiment of the present invention is a ***FcR*** that comprises a mutein that binds to a ***Fc*** domain of an antibody. Such a mutein has an improved function compared to a protein comprising SEQ ID NO:2. Examples of such an improved function include, but are not limited to, increased stability, increased affinity for an ***Fc***

domain of an antibody, altered substrate specificity, and increased solubility. Such a mutein can be produced by a method that. . . disrupt the 3-D structure of the protein; i.e., the modified protein, or mutein, is still capable of binding to the ***Fc*** domain of an antibody. A preferred mutein is a ***FcR*** that binds to a Fe domain of an IgE antibody, although the invention also covers muteins binding to other classes. . .

DETD . . . concentrations, to oxidation and/or reduction, to deamidation, to other forms of chemical degradation and to proteolytic degradation compared to unmodified ***FcR*** . Increased stability can also refer to the ability of a mutein of the present invention to be stable for a. . . thiocyanate, etc. A preferred mutein of the present invention has a melting temperature substantially higher than that of an unmodified ***FcR*** . Preferably the melting temperature of a mutein is at least about 1.degree. C. higher, and more preferably at least about. . .

DETD [0065] Another embodiment of the present invention is a mutein that exhibits increased affinity for a ***Fc*** domain of an antibody compared to its unmodified counterpart. As used herein, a mutein having increased affinity is a ***FcR*** that exhibits a higher affinity constant (K.sub.A) or lower dissociation constant (K.sub.D) than its unmodified counterpart. Such a higher affinity constant can be achieved by increasing the association rate (k.sub.a) between the mutein and the ***Fc*** domain and/or decreasing the dissociation rate (k.sub.d) between the mutein and the ***Fc*** domain. A preferred mutein of the present invention has a K.sub.A for a ***Fc*** domain of at least about 3.times.10.sup.9 liters/mole (M.sup.-1), which is equivalent to a K.sub.D of less than or equal to about 3.3.times.10.sup.-10 moles/liter (M). More preferred is a mutein having a K.sub.A for a ***Fc*** domain of at least about 2.times.10.sup.10 M.sup.-1, and even more preferably of at least about 1.times.10.sup.11 M.sup.-1. Also preferred is a mutein having a k.sub.a for a ***Fc*** domain of at least about 1.times.10.sup.5 liters/mole-second as well as a mutein having a k.sub.d for a ***Fc*** domain of less than or equal to 3.times.10.sup.-5/second. More preferred is a mutein having a k.sub.a for a ***Fc*** domain of at least about 3.times.10.sup.5 liters/mole-second, and even more preferably of 1.times.10.sup.6 liters/mole-second. Also preferred are muteins having a k.sub.d for a ***Fc*** domain of less than or equal to 1.times.10.sup.-5/second or even more preferably less than or equal to 3.times.10.sup.-4/second. A preferred ***Fc*** domain is that of an IgE antibody. Methods to measure such binding constants is well known to those skilled in the art; see, for example, Cook et al., 1997, ibid., which reports the following values for the binding of ***human*** ***Fc*** .epsilon.RI.alpha. protein to ***human*** IgE: k.sub.a1 of 3.5 (.+-.0.9).times.10.sup.5 M.sup.-1s.sup.-1; k.sub.a2 of 8.6 (.+-.3.5).times.10.sup.4M.sup.-1s.sup.-1; k.sub.d1 of 1.2 (.+-.0.1).times.10.sup.-2s.sup.-1; k.sub.d2 of 3.2 (.+-.0.8).times.10.sup.-5s.sup.-1; K.sub.A1 of. . .

DETD . . . to its unmodified counterpart. A mutein exhibiting altered substrate specificity is a mutein that binds with increased affinity to a ***Fc*** domain of an antibody class or antibody species of a different type than that normally bound by its unmodified counterpart. In one embodiment, a mutein of a ***human*** ***Fc*** .epsilon.RI.alpha. protein with altered substrate specificity is a ***FcR*** that binds with increased affinity to a IgE antibody of another mammal, such as, but not limited to, a canine, feline, equine, murine, or rat IgE antibody. In another embodiment, a mutein of a ***human*** ***Fc*** .epsilon.RI.alpha. protein with altered substrate specificity is a ***FcR*** that binds with increased affinity to an antibody of another class, such as IgG, IgM, IgA, or IgD, with IgG. . .

DETD [0068] As disclosed herein, the 3-D model representing a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 complex is advantageous in determining strategies for producing muteins having an improved function, e.g., for identifying targets to modify in order to obtain muteins having improved functions. Examples of targets are as follows. A key feature of the ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein is the crystal contacts in five space groups, a subset of which are predicted to interact directly with a ***Fc*** domain of an IgE antibody. Such contacts are included in the IgE binding domain which is unique for ***human*** ***Fc***

.epsilon.RI.alpha. in that the domain includes a tryptophan-containing hydrophobic ridge positioned on the top face of the crystal structure (i.e., amino. . . 147-155 of SEQ ID NO:2. Yet another striking feature is the finding that the amino and carboxyl termini of the ***human*** ***Fc*** .epsilon.RI.alpha..sub.1-176 protein are only 10 angstroms apart. Particularly preferred targets are a crystal contact cluster, a tryptophan-containing hydrophobic ridge, a FG. . . of D2, the amino terminal five residues of said protein, and the carboxyl terminal five residues of said protein, with ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1, a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 2, a C strand of domain 2 of ***Fc8RI*** .alpha., a C'E loop of domain 2 of ***Fc*** .epsilon.RI.alpha., and a tryptophan-containing hydrophobic ridge of ***Fc*** .epsilon.RI.alpha. being particularly preferred. Preferred residues to target include residues at positions 85, 86, 87, 110, 113, 117, 119, 126,129,130, 131,. . . regions to target are listed in Tables 3, 4, and 5.

TABLE 3

Contact analysis between specified sets of atoms in ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1

set1 = (segid A)
set2 = (segid B)
definition of contact atoms: (known and not hydrogen. . .
DETD [0069]
TABLE 4

Contact analysis between specified sets of atoms in ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1

set1 = (segid A)
set2 = (segid D)
definition of contact atoms: (known and not hydrogen. . .
DETD [0070]
TABLE 3

Contact analysis between specified sets of atoms in ***Fc*** .epsilon.RI.alpha.-CHAPs interaction

set1 = (segid A)
set2 = (segid E)
definition of contact atoms: (known and not hydrogen)
maximum distance. . .
DETD . . . present invention to produce the mutein having the improved function. Knowledge of the structure of the extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein crystal, for example, permits the rational design and construction of modified forms of the protein by permitting the prediction. . . bridges, hydrophobic interactions and hydrogen bonds unless the goal is to purposefully change such interactions. The 3-D structure of the ***human*** ***Fc*** .epsilon.RI.alpha. protein suggests that large deletions may not be desirable, particularly due to the relation between the various domains of the. . .
DETD . . . a method to produce a mutein includes the steps of (a) comparing a key region of a model of a ***human*** ***Fc*** .epsilon.RI.alpha. protein with the amino acid sequence of a ***FcR*** having an improved function compared to the unmodified ***Fc*** .epsilon.RI.alpha. protein in order to identify at least one amino acid segment of the ***FcR*** with the improved function that if incorporated into the ***Fc*** .epsilon.RI.alpha. protein represented

by the model would give the ***Fc*** .epsilon.RI.alpha. protein the improved function; and (b) incorporating the segment into the ***Fc*** .epsilon.RI.alpha. protein, thereby providing a mutein with the improved function. In another embodiment, a method to produce a protein includes the steps of: (a) using a model representing a ***human*** ***Fc*** .epsilon.RI.alpha. protein to identify a 3-D arrangement of residues that can be randomized by mutagenesis to allow the construction of a. . . a method that includes the steps of: (a) effecting random mutagenesis of nucleic acid molecules encoding a target of a ***Fc*** .epsilon.RI.alpha. protein as identified by analyzing a model of that protein, such as an IgE binding domain; (b) cloning such mutagenized. . .

DETD . . . at least one amino acid in at least one non-constrained loop of domain 1 in an area proximal to the ***FceRI*** gamma chain putative binding site; (b) joining an amino-terminal amino acid residue to a carboxyl-terminal amino acid residue of an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein; (c) replacing at least one amino acid site with an amino acid suitable for derivatization; (d) replacing at least. . .

DETD . . . or chemical synthetic methods of a nucleic acid molecule encoding the desired protein, such as, but not limited to a ***human*** ***Fc*** .epsilon.RI.alpha. protein, followed by expression of the mutated gene in a suitable expression system, preferably an insect, mammalian, bacterial, yeast, insect,. . .

DETD . . . present invention is a mutein in which at least one amino acid in at least one non-constrained loop of a ***Fc*** .epsilon.RI.alpha. protein is replaced in order to improve a function of the protein. Finding that the ***human*** ***Fc*** .epsilon.RI.alpha. protein had such loops was surprising, and it is believed, without being bound by theory, that a mutein in which. . . domain 1 (i.e., spanning amino acids 31-35 and 70-74 of SEQ ID NO:2), preferably in an area proximal to the ***FceRI*** gamma chain putative binding site, i.e., the site on the ***Fc*** .epsilon.RI.alpha. protein to which the gamma chain of the high affinity ***Fc*** ***epsilon*** ***receptor*** is thought to bind. In a preferred embodiment, one or more amino acids is replaced to make loops shorter, but. . .

DETD [0078] Another embodiment of the present invention is a mutein of the extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein in which an N-terminal (amino-terminal) amino acid residue is joined, preferably covalently, to a C-terminal (carboxyl-terminal) amino acid residue in order to improve a function of the protein. Finding that the N-termini and C-termini of the ***human*** ***Fc*** .epsilon.RI.alpha. protein were only 10 angstroms apart was quite surprising. Without being bound by theory, it is believed that such a. . .

DETD . . . site, is replaced with a charged or polar residue to increase solubility or create more stable muteins. Glycosylation sites in ***human*** ***Fc*** .epsilon.RI.alpha.. protein include amino acids 21, 42, 50 74, 135, 140, and 166 of SEQ ID NO:2. A preferred amino acid. . .

DETD . . . order to improve the function of the mutein, at least by increasing stability. Cysteine pairs can be substituted into a ***Fc*** .epsilon.RI.alpha. protein at any two residue positions identified with available programs and algorithms that would allow the formation of an undistorted. . .

DETD . . . N-linked glycosylation sites are added to or removed from the protein, preferably by substitution with an appropriate amino acid. A ***Fc*** .epsilon.RI.alpha. protein with additional N-linked glycosylation sites is more soluble. The ability to design a ***Fc*** .epsilon.RI.alpha. protein having fewer, or no, N-linked glycosylation sites is also valuable as production of such a protein from production run to production run is likely to be more uniform. One embodiment is a ***Fc*** .epsilon.RI.alpha. mutein with no N-linked glycosylation sites that is stable, active, and soluble. Such a protein has an advantage of being. . .

DETD [0091] Another embodiment of the present invention to enhance stability is the addition of polyethylene glycol (PEG) groups to a ***FcR*** protein, i.e., to produce a "pegylated" ***FcR*** protein. In one embodiment, the PEG group(s) can substitute for carbohydrate group(s) due to removal of one or more N-glycosylation. . .

DETD [0092] Another embodiment of the present invention is a mutein that

comprises a ***FcR*** having a substance, such as a ligand or ***detectable*** ***marker*** , attached to an amino acid of the protein such that the substance does not substantially interfere with the antibody binding. . . its function, such as binding to a second member of a ligand pair or enabling detection of the protein. The ***FcR*** to which a substance is attached can be either an unmodified protein or a mutein of the present invention. Suitable. . . nature of the amino acid prior to any modification required for attachment. Examples of suitable substances to attach to a ***FcR*** include any compound capable of binding to or reacting with another substance, such as those described for attachment to a. . .

DETD . . . to expand the use of models of the present invention to produce models of and make modifications to any suitable ***FcRs*** or other Ig domain-containing proteins to produce muteins having a desired function.

DETD [0095] The present invention also includes a mutein that binds to an IgE binding domain of a ***Fc*** .epsilon.RI.alpha. protein, wherein the mutein has an improved function compared to a ***Fc*** -C.epsilon.3/C.epsilon.4 protein comprising amino acid sequence SEQ ID NO:6. Such an improved function can include increased stability compared to the stability of a ***human*** IgE ***Fc*** region comprising amino acid sequence SEQ ID NO:6, increased affinity for a ***Fc*** .epsilon.RI.alpha. protein compared to the ***Fc*** .epsilon.RI.alpha. affinity of a ***human*** IgE ***Fc*** region comprising amino acid sequence SEQ ID NO:6, altered substrate affinity compared to the affinity for ***human*** ***Fc*** .epsilon.RI.alpha. of a ***human*** IgE ***Fc*** region comprising amino acid sequence SEQ ID NO:6, and increased solubility compared to the solubility of a ***human*** IgE ***Fc*** region comprising amino acid sequence SEQ ID NO:6. Such a mutein is produced by a method that includes the steps. . . three-dimensional model substantially representing the atomic coordinates specified in Table 1 to identify at least one amino acid of the ***Fc*** -C.epsilon.3/C.epsilon.4 protein represented by said model which if replaced by a specified amino acid would effect said improved function of said ***Fc*** -C.epsilon.3/C.epsilon.4 protein; and (b) replacing said identified amino acid(s) to produce said mutein having said improved function. ***Fc*** muteins can be identified and produced in a manner similar to that described herein for ***FcR*** muteins. Antibody muteins have a variety of uses, including but not limited to, diagnostic and therapeutic uses. For example, muteins. . . express an antibody receptor protein using, for example, radioimmune therapy of derivatized IgE. Muteins could also be used for monitoring ***FcR*** expression in atopic individuals (e.g. with a tag for one-step FACS analysis) or for monitoring IgE in atopic individuals. Muteins could also be used as inhibitors or as toxin-IgE- ***Fc*** fusion proteins to target ***FcR*** -expressing cells to kill them (e.g. in mast cell tumors or severe allergy). Also muteins that affect the low affinity affinity IgE-receptor (***FceRII***) binding but not ***FceRI*** binding could be designed or selected.

DETD . . . novel structures as identified by a 3-D model of the present invention. Preferred structures exhibiting direct interaction between IgE and ***Fc*** .epsilon.RI.alpha. include ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1, a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 2, a C strand of domain 2 of ***Fc*** .epsilon.RI.alpha., a C'E loop of domain 2 of ***Fc*** .epsilon.RI.alpha., and a tryptophan-containing hydrophobic ridge of ***Fc*** .epsilon.RI.alpha.. Other preferred structures include a crystal contact cluster involved in IgE binding; a FG loop in D2; a D1D2 interface;. . . a BC loop of D2; a CC' loop of D2; and a strand of D2. Particularly preferred are (a) a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1 pocket comprising an amino acid residue at position 131 of SEQ ID NO:2 and amino acid residues at positions 9, 11, 37, 39, and 99 of SEQ ID NO:6 and (b) a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 2 pocket comprising amino acid residues at positions 85, 86, 87, and 110 of SEQ ID NO:2 and. . .

DETD [0107] The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a ***FcR*** or antibody of the present invention. As used herein, the term

"selectively binds to" refers to the ability of antibodies. . .

DETD [0109] A ***FcR*** of the present invention can include chimeric molecules comprising at least a portion of a ***FcR*** that binds to an antibody and a second molecule that enables the chimeric molecule to be bound to a substrate. . . a manner that the antibody receptor portion binds to the antibody in at least as effective a manner as a ***FcR*** that is not bound to a substrate. An example of a suitable second molecule includes a portion of an immunoglobulin. . .

DETD . . . entireties. It is to be noted that although the compositions and methods disclosed in WO 98/27208, ibid., relate to feline ***Fc*** .epsilon.RI.alpha. proteins, they are also applicable to therapeutic compositions of the present invention. Therapeutic compositions of the present invention are advantageous. . .

DETD . . . entireties. It is to be noted that although the reagents and methods disclosed in WO 98/27208, ibid., relate to feline ***Fc*** .epsilon.RI.alpha. proteins, they are also applicable to diagnostic reagents, kits and detection methods of the present invention. Muteins of the present. . .

DETD . . . production and analysis of a crystal and model of the present invention. It is to be noted that numbering of ***Fc*** -C.epsilon.3/C.epsilon.4 residues follows the convention of Dorrington et al, ibid.

DETD [0115] The initiation of IgE-mediated allergic responses requires the binding of IgE antibody to its high affinity receptor, ***Fc*** .epsilon.RI. Crosslinking of ***Fc*** .epsilon.RI initiates an intracellular signal transduction cascade that triggers the release of mediators of the allergic response. The interaction of IgE- ***Fc*** domains with ***Fc*** .epsilon.RI is a key recognition event that is central to this process and mediated by the extracellular domains of the .alpha.-chain of ***Fc*** .epsilon.RI. This Example describes the solution of a crystal structure of the ***human*** IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex, the coordinates of which are disclosed in Table 1. The crystal structure reveals that one receptor binds one IgE- ***Fc*** asymmetrically through interactions at two sites involving both N-terminal IgE- ***Fc*** C.epsilon.3 domains. The interaction of one receptor with IgE- ***Fc*** blocks the high-affinity binding of a second receptor and features of this interaction are conserved in other ***Fc*** receptor family members. The structural analysis suggests new approaches to the inhibition of IgE binding to ***Fc*** .epsilon.RI for the treatment of allergy and asthma.

DETD [0117] The high affinity IgE receptor (***Fc*** .epsilon.RI) is found on the surface of effector cells of the immune system that initiate cellular reactions associated with the allergic response, anaphylaxis and anti-parasitic immunity.sup.1,2. The ***human*** receptor can form either a trimeric .alpha..gamma..sub.2 or tetrameric .alpha..beta..gamma..sub.2 structure on cell surfaces, with the extracellular domains of the. . . triggered through a similar activation of eosinophils, leading to the release of granular proteins toxic to schistosomes and other parasites. ***Fc*** .beta.RI belongs to a family of antibody-binding receptors that also mediate interactions of soluble IgG and IgA antibodies with cells of the immune system.sup.3,5. IgG- ***Fc*** receptors regulate inflammation pathways, B cell development, and Natural Killer Cell activation and are therefore important in many aspects of. . .

DETD . . . associated with the IgE network through genetic studies in both mice and humans, suggesting a role for polymorphisms of the ***Fc*** .epsilon.RI .beta.-chain and CD14 in atopic individuals.sup.7,8. The interaction of the IgE antibody with ***Fc*** .epsilon.RI is central to these immune reactions, providing an attractive target for the inhibition of all IgE-mediated allergic disease. Clinical studies. . . approach to disease treatment.sup.9,10. Further development of treatments for allergy, asthma and anaphylaxis, may benefit from structural insights into the IgE: ***Fc*** .epsilon.RI interaction.

DETD [0119] A recent report disclosed the crystal structure of the ***human*** ***Fc*** .epsilon.RI .alpha.-chain ectodomains.sup.11, which revealed a highly bent arrangement of two immunoglobulin domains. Four solvent-exposed tryptophans cluster at the top of the receptor, forming a large hydrophobic surface for potential interactions with the IgE- ***Fc*** . This tryptophan cluster borders the ***Fc*** binding-site mapped by mutagenesis studies, which implicate residues in

the second domain of the receptor in IgE binding. The structural. . .
DETD [0120] These questions are addressed with the solution of a crystal structure of a complex of the ***human*** IgE- ***Fc*** with ***Fc*** .epsilon.RI as disclosed herein as well as of a crystal structure of the unbound IgE- ***Fc*** fragment as disclosed in 60/189,403, ibid. The structure of the complex reveals two interaction sites for the IgE- ***Fc*** on the receptor surface and clarifies how a 1:1 complex between antibody and receptor is formed. The two IgE- ***Fc*** C.epsilon.3 domains bind to distinct sites on the receptor; one is formed by the C-C' loop in the receptor D2. . . the C.epsilon.3 interaction sites. The structure of the complex accounts for previous mutagenesis and structural observations and shows that the ***Fc*** forms a complementary crown across the convex surface of the receptor. Comparison of the complex with the isolated IgE- ***Fc*** crystal structure suggests that large structural changes may occur upon IgE binding to its receptor (see No. 60/189,403, ibid.) The IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex provides a model for understanding the function of other antibody ***Fc*** -receptors and new conceptual approaches to the inhibition of IgE-mediated diseases.
DETD [0122] The crystallization of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex required the expression of each protein using recombinant baculovirus technology. The expression of the ***Fc*** .epsilon.RI.alpha. was carried out essentially as described previously.sup.11. The IgE heavy chain contains four constant domains (C.epsilon.1-C.epsilon.4), in contrast to the three found in IgG antibodies. The interaction of ***Fc*** .epsilon.RI with IgE has been previously mapped to the two C-terminal constant domains of the IgE- ***Fc*** (domains C.epsilon.3/C.epsilon.4).sup.12-16. The expression and purification of the ***human*** IgE- ***Fc*** C.epsilon.3/C.epsilon.4 domains was established as described (No. 60/189,403, ibid.) and purified protein used to form complexes with ***Fc*** .epsilon.RI.alpha.. The best complex crystals (spacegroup P42.sub.12) obtained with the wild type (wt) ***Fc*** .epsilon.RI.alpha. protein were small (.about.60-100 .mu./edge) and diffraction data was limited to a resolution of .about.4.5 .ANG. (Table 3, crystal form I). In order to improve the complex crystals, a triple carbohydrate mutant of ***Fc*** .epsilon.RI.alpha. (***Fc*** .epsilon.RI.alpha..DELTA.4-6) was expressed in insect cells. The ***Fc*** .epsilon.RI.alpha..DELTA.4-6 mutant lacks carbohydrate at three of the seven native attachment sites (residues 74, 134, 140) and was previously shown to produce .about.50% of the wt protein in CHO cells.sup.17. Complexes formed with baculovirus-expressed ***Fc*** .epsilon.RI.alpha..alpha.4-6 grow crystals in spacegroup R32 and diffract X-rays to a resolution of 3.25 .ANG. (Table 3, crystal form II). The. . . an overall R-free of 29.3% and R-cryst of 27.0% to 3.25 .ANG.. FIG. 1a shows electron density from a sigmaa-weighted 2Fo- ***Fc*** simulated annealing omit map calculated with the current model phases.
DETD [0124] Both crystal forms of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex contain a single 1:1 complex in the asymmetric unit, with similar overall geometric features (FIGS. 1b, c). Given the. . . the interfaces is limited to crystal form II. Binding interactions are formed exclusively between the N-terminal C.epsilon.3 domains of the IgE- ***Fc*** with ***Fc*** .epsilon.RI.alpha.. The C.epsilon.4 domains of the IgE- ***Fc*** point away from the receptor structure and make no contacts with either receptor domain. The C.epsilon.3/C.epsilon.4 hinge regions are also. . . the C.epsilon.3 domains (FIGS. 1b,c). The angle between the C.epsilon.3 and C.epsilon.4 domains also differs from that seen in the IgE- ***Fc*** alone (see 60/189,403, ibid.) While structured carbohydrate is visible in both the IgE- ***Fc*** and ***Fc*** .epsilon.RI.alpha. proteins, the carbohydrate groups do not contribute significantly to interactions between the two molecules. In addition, the IgE- ***Fc*** carbohydrate does not make any contacts across the IgE- ***Fc*** diad axis, but lies along the surface of each IgE- ***Fc*** domain.
DETD [0125] The IgE-C.epsilon.3 domains bind at the top of the ***Fc*** .epsilon.RI.alpha. D1/D2 interface and along the backside of the D2 domain. The receptor contains two distinct binding sites for the two. . . the top of the receptor and involves four surface-exposed tryptophans (W87, W110, W113, and W156). The two chains of the ***Fc*** molecule bind the receptors using surface loops in C.epsilon.3 that are distal to